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Activities of Repository Preparations of Cycloguanil Pamoate and 4,4′-Diacetyldiaminodiphenylsulfone, Alone and in Combination, Against Infections with *Plasmodium cynomolgi* in Rhesus Monkeys†

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The studies summarized in this report were concerned with the capacities of repository preparations of cycloguanil pamoate (CGT-P) to protect rhesus monkeys against infections with drug-susceptible and pyrimethamine-resistant strains of *Plasmodium cynomolgi*. Administered intramuscularly as a suspension in an oleaginous vehicle, CGT-P (i) provided long-term protection against single and repetitive challenges of rhesus monkeys with sporozoites of the drug-susceptible *B* and *Ro* strains, (ii) effected prompt clearance of parasitemia in established infections, and (iii) delayed relapse. Protection was equated to absence of parasites on thick blood films, negative results when blood was transferred to susceptible recipients, and inability to activate infection by splenectomy. Eventual loss of protection was not related to emergence of parasites resistant to cycloguanil (CGT). Although protection varied from monkey to monkey, its mean duration was related directly to size of CGT-P dose and size of particles in the suspension. Urinary excretion studies indicated that protection persisted as long as the daily output of CGT did not fall below that attained with the parenterally administered hydrochloride salt at a dose equivalent to 0.015 mg of CGT per kg. Studies on infections with the resistant *Ro/PM* strain showed that the activity of CGT-P was compromised severely by resistance to pyrimethamine. Attempts to minimize this liability by concomitant administration of 4,4′-diacetyldiaminodiphenylsulfone met with limited success. These results suggest that even the best of the repository preparations of CGT-P, with or without 4,4′-diacetyldiaminodiphenylsulfone, would be useful only in areas where *Plasmodium falciparum* and *Plasmodium vivax* are fully susceptible to chlorguanide and pyrimethamine.

The studies recorded in this report were initiated in 1961 and completed in 1966. They constituted the central part of a collaborative effort aimed at meeting the need (4) for a long-lasting antimalarial drug that could be used in consort with residual insecticides in malaria eradication programs (65). This effort was catalyzed by the late Paul Thompson of the Research Division, Parke, Davis & Co., Ann Arbor, Mich., and rested on the results of his studies on the activities of repository preparations of cycloguanil pamoate (CGT-P) (the pamoic acid salt of the triazine metabolite of chlorguanide [CGT]), 4,4′-diacetyldiaminodiphenylsulfone (DADDS), and combinations of CGT-P and DADDS against infections with trophozoites of *Plasmodium berghei* in mice and *Plasmodium cynomolgi* in monkeys (60, 61). Our studies, employing infections with sporozoites of various strains of *P. cynomolgi* in rhesus monkeys as the primary experimental tool, dealt in sequence with (i) the capacity of CGT-P to provide long-term protection against infections with drug-susceptible strains of this plasmodium and with the major determinants of that capacity; (ii) measurements of the release of CGT from the muscle depot of CGT-P and how the dynamics of this release affected the duration of protection; (iii) the impacts of preexisting resistance to pyrimethamine on protection accorded by CGT-P; and (iv) the capacity of DADDS, delivered in combination with CGT-P, to limit the liabilities of pyrimethamine resistance.

The results of the above studies provided the underpinning for assessments of the activities of CGT-P and combinations of CGT-P and DADDS against infections with sporozoites of *Plasmodium vivax* and *Plasmodium falciparum* in human volunteers (8–10, 12, 13, 17, 31, 38, 39). It had been anticipated that the results of these appraisals, together with the results of studies on infections with *P. cynomolgi*, would guide the design and execution of field trials. Unfortunately, this was not the case. Impelled by the successes of the early studies in human volunteers (12, 13, 17, 31), field studies, first on CGT-P alone and later on this agent combined with DADDS, were undertaken in West Pakistan (15), New Guinea (40, 41), Australia (1, 2), West Africa (21, 25, 28, 32–37). East Africa (11, 29), and Brazil (24), with scant attention to factors then recognized as determinants of the efficacies of these agents. Predictably, results fell far short of expectations, leading by 1968 to almost total loss of interest in further explorations of the potentials of CGT-P alone or in combination with DADDS.

Interest in long-lasting antimalarial drugs was renewed in 1976, catalyzed and supported by the UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases and the U.S. Army Medical Research and Development Command (WHO-TDR/CHEMAL/SC(33)/77.3, item 1.2, p. 1). The principal focus of these studies has been on protection accorded against infections with trophozoites of *P. berghei* by subcutaneous implants of biodegradable polymers containing pyrimethamine, CGT, a quinazoline designated WR-158,122, or sulfadiazine. Reviews of both published and informal reports (26,

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27, 62–64; WHO-TDR/PR-6/83.2-MAL, item 2.15, p. 25) have indicated that continued pursuit of these investigations would benefit from access to hitherto unpublished results of the aforementioned studies on the activities of CGT-P alone and in combination with DADDS against infections with both drug-susceptible and pyrimethamine-resistant strains of *P. cynomolgi*. This report is concerned with the conduct, results, and implications of the major and as yet unpublished segments of these investigations.

MATERIALS AND METHODS

Since many of the technical procedures used in the series of experiments recorded in this report were tailored to the objectives of individual studies, it has seemed best to detail these special methods along with the results of the experiments that they served. Only the more generally used materials and methods will be described in this section.

Test compounds, preparations for use, and methods of administration. Four lots of CGT-P (8652, 4699-X74, 9163, and 590647), one lot of DADDS (9029), and one lot of CGT hydrochloride (446OX16), all provided by Paul Thompson, Research Division, Parke, Davis & Co., were used in the studies included in this report. The lots of CGT-P differed from each other with respect to particle size. Lot 8652, with particles ranging from 1 to 105 μ in greatest dimensions, with 70% in excess of 50 μ, was categorized as a large-sized particle preparation. Lots 4699-X74, 9163, and 590647, with respective particle sizes of 10 to 40, 10 to 50, and 25 to 50 μ, were categorized as medium-sized particle preparations. The single lot of DADDS, made up of particles averaging 36 μ in greatest dimensions, was prepared to match the medium-sized particle lots of CGT-P.

Lot 8652 of CGT-P, the first and most frequently used preparation in our studies, was provided in 5-ml glass vials as a sterile suspension with the equivalent of 150 mg of CGT base per ml in a 40% benzyl benzoate–60% castor oil vehicle. Lots 4699-X74, 9163, and 590647 of CGT-P were provided as bulk crystalline powders, as were DADDS and CGT hydrochloride. The quantity of such a bulk preparation approximately 20% larger than that required for dosing all monkeys in a specific experiment was autoclaved in a sealed vial, under conditions that assured maintenance of chemical integrity (60), and then transferred to an Erlenmeyer flask with a Teflon-lined screw cap and suspended by intensive shaking in either sterile benzyl benzoate-castor oil or aqueous vehicle. The latter, also provided by Thompson, was a proprietary mixture of dispersing agents with polysaccharides to impart viscosity and was used only with lot 4699-X74 of CGT-P. Stock suspensions of CGT-P containing the equivalent of 150 mg of CGT per ml met the largest dose needs of all experiments in which this compound was administered alone. Suspensions containing 300 mg of CGT per ml were required when this agent was administered in combination with DADDS. Stock suspensions of DADDS containing the equivalent of 300 mg of dapsone per ml were required in some studies involving both single and combination agent regimens. Stock suspensions of CGT-P plus DADDS were prepared by blending the appropriate volumes of the stock suspensions of the individual agents.

Irrespective of dose, suspensions of CGT-P alone, DADDS alone, or combinations of these agents were administered in a volume of 0.5 ml/kg of body weight. This volume was achieved by diluting the appropriate amounts of the stock suspensions with either the oleaginous or the aqueous vehicle. CGT-P, DADDS, and their combinations were injected into the gluteal muscle mass of the flexed right thigh of the monkey. Special attention had to be given to the injection procedure because of the viscosity of the suspending medium and the tendency of particles of the test compounds to settle out, freeze syringe plungers, and clog needle orifices. The procedure developed to eliminate this problem was important to the atraumatic and quantitative administration of the test agents; hence, it merits detailed description here. All preparations, irrespective of agent concentration, were shaken vigorously and continuously in a 50°C bath for at least 30 min before syringe loading and injection. Luer-Lok 2-ml glass syringes (Becton Dickinson and Co., Paramus, N.J.) fitted with 2.54-cm, short-bevel, 20-gauge needles, were kept at 50°C until they were loaded with the appropriate volume of test compound. Immediately after loading, this volume was injected into the muscle mass, a process that took no more than 90 s. By having everything in place and following this procedure rigidly, freezing of the syringe and accumulation of test agent in the syringe base and needle hub rarely occurred. When it did, the affected monkey was removed from the experiment.

CGT hydrochloride was always administered via stomach tube by techniques detailed heretofore (48). The quantities of compound required for individual monkeys were drawn from freshly prepared stock solutions containing 1 mg of CGT per ml of distilled water.

Monkeys. The data assembled in this report were based on studies on 677 feral rhesus monkeys (*Macaca mulatta*) imported directly from New Delhi, India. Approximately equal numbers of males and females were included in this total. At the time of assignment to an experiment, 550 of the subjects weighed between 2.0 and 4.0 kg and 127 weighed between 4.2 and 6.5 kg. The procedures for acquiring these monkeys and transporting them from India to Cincinnati, Ohio, or Davis, Calif., the methods of conditioning the newly imported animals for experimental use, the routine colony husbandry practices (including dietary management), and the handling procedures employed in all facets of the malaria studies were identical with those described previously (49).

Of the 677 monkeys, 436 (327 treated, 109 untreated) were infected with sporozoites and 47 (42 treated, 5 untreated) were infected with trophozoites. Of the remaining 194, 167 were recipients of blood drawn at various times after sporozoite challenge from monkeys treated with CGT-P or DADDS. The remaining 27 were used in studies on the rate of release of CGT from depots of CGT-P in muscle.

Parasitological elements. The drug-susceptible *B* and *Ro* strains and the highly pyrimethamine-resistant *Ro/PM* strain of *P. cynomolgi* were used in these studies. The origins of these strains, the monkey-to-mosquito-to-monkey and the monkey-to-monkey passage procedures used to maintain them, the characteristics of untreated infections induced with either sporozoites or trophozoites of these strains, and the responses of such infections to standard antimalarial drugs have been detailed and documented elsewhere (50, 53). Of the 483 monkeys committed to primary assessments of prophylactic and therapeutic activities, 214 were inoculated with sporozoites and 27 with trophozoites of the *B* strain, 44 with sporozoites of the *Ro* strain, and 178 with sporozoites and 20 with trophozoites of the *Ro/PM* strain.

Sporozoites required for primary inoculation and rechallenge purposes were derived from the ground thoraces of mosquitoes (*Anopheles freeborni*) that had fed 13 to 15 days previously on monkeys in the appropriate monkey-to-mosquito-to-monkey passage line. The current studies were served by 69 lots of infected mosquitoes: 57 with the *B*

strain, 4 with the *Ro* strain, and 8 with the *Ro/PM* strain. Dissections of 10 mosquitoes per lot, performed 5 to 7 days after monkey feeding, showed that the above lots were 90 to 100% gut positive, with an average of 20 to >100 oocysts per stomach. Dissections on 5 to 10 mosquitoes 7 days after gut dissection revealed large numbers of sporozoites in salivary glands. When processed for challenge purposes 13 to 15 days after monkey feeding, these lots provided inocula ranging from 1.8×10^4 to 9.6×10^6 sporozoites for each of 12 to 60 monkeys in an experiment. Such inocula, in 1 ml of 50:50 monkey serum-saline, were injected intravenously. The diverse procedures used to (i) identify the phase of the infection in the passage monkey when mosquito feedings were most likely to lead to appropriately infected insects, (ii) feed lots of 500 to 1,000 mosquitoes on monkeys in the passage lines, (iii) assess the density of gut infections and intensity of salivary gland infections in such lots, (iv) prepare sporozoite suspensions from mosquito thoraces and enumerate the numbers of sporozoites therein, and (v) inoculate sizeable groups of monkeys intravenously with the requisite numbers of sporozoites all of the same viability were identical with procedures described previously (50, 52).

Inocula for trophozoite-induced infections were derived from animals in the monkey-to-monkey passage lines. An inoculum of 5×10^5 parasites injected intravenously was used in all studies. Preparation of such inocula has been described elsewhere (49).

The procedures used to detect and quantify parasitemias on thick and thin blood films and the schedules followed in preparing and examining these films during the progressive phases of the malaria infection were identical with those detailed previously (49). There was but one break in the schedule of blood film examinations during the entire project. This involved chronically infected monkeys only and occurred between 2 May and 18 June 1963, subsequent to shipment of infected monkeys from Cincinnati, Ohio, to Davis, Calif., and before completion of quarters that permitted handling of the animals on a regular schedule.

There were 167 subinoculations of blood from inoculated monkeys to previously unchallenged monkeys. This procedure, the most sensitive of all methods other than splenectomy for detection of low-level parasitemias (49), was employed in situations in which it was important to determine whether an infection was patent despite the failure to detect parasites via repetitive, rigorous searches of thick blood films. It involved withdrawal of 10 to 15 ml of blood from the antecubital vein of the donor monkey into a 20-ml glass syringe containing 1 ml of 5% sodium citrate, mixing the blood with the citrate in the syringe, and immediately reinjecting the contents of the syringe through a fresh needle into the mid-saphenous vein of a recipient monkey. Examinations of blood films of the latter subject were initiated the day after blood transfer and were continued until parasitemia was patent or for at least 30 days. If films were consistently negative to the end of that observation period, the recipient monkey was treated via stomach tube with a curative course of chloroquine (5.0 mg/kg daily for 4 consecutive days) and returned to the stock colony for reuse in other than malaria studies.

Splenectomy, as a test for total eradication of parasites (49), was performed in 38 instances. Surgery was carried out under sodium pentobarbital anesthesia, with rigid attention to aseptic technique. Thick blood films were prepared and examined daily for at least 21 days after splenectomy. If films were uniformly negative over this study period, the splenectomized monkey was rechallenged with 6.9×10^4 to 3.4×10^6 sporozoites of the homologous strain of *P. cynomolgi* and subjected to a series of blood film examinations identical with those employed in delineating response to the initial sporozoite challenge.

RESULTS

Activities of CGT-P against infections with the *B* strain. (i) Efficacy against repeated sporozoite challenges. This study involved work with 74 monkeys: 15 dosed with CGT-P and challenged 3 to 11 times with sporozoites, 30 untreated controls for these challenges, and 29 recipients of blood drawn at various times during interchallenge intervals from 9 of the 15 subjects given CGT-P. The dose of this agent, the large-sized particle lot, was equivalent to 50.0 mg of CGT per kg of body weight. Of the 15 recipients of CGT-P, 12 were first challenged 7 days after dosage and 3 were challenged at 21 days. These 3 and 10 of the 12 challenged at 7 days after dosage were rechallenged at 27- to 35-day intervals until infections were established. Departures from the planned 28-day rechallenge schedule were dictated by the times when lots of heavily infected mosquitoes were available. The remaining 2 monkeys of the group of 15 were rechallenged the first and second times 306 and 386 days after the initial challenge. Subinoculations, limited to the 10 monkeys first challenged 7 days after CGT-P dosage and rechallenged at 27- to 35-day intervals thereafter, were initiated after the seventh sporozoite challenge and were performed on the day on which the untreated control monkey for the rechallenged group exhibited a patent parasitemia.

As evidenced by the absence of microscopically demonstrable parasitemia, the dose of CGT-P used in this study provided protection against repeated challenges with sporozoites for at least 180 days and, in one case, for at least 537 days (mean, 277 days) (Table 1, column 3). Of the 15 recipients of the above dose, 10 were protected for 245 days or longer. The time to appearance of parasitemia after the last challenge varied considerably, from 8 to 9 days (as in untreated controls) to 32 to 35 days (Table 1, column 5). The latter delays in onset of patency, just short of the time for the next sporozoite challenge, doubtless reflected the sustained outflow of CGT from the CGT-P depot in amounts sufficient for suppression of parasitemia.

The results of the subinoculation studies (Table 1, column 4) show that only four of the nine transfers of blood carried out on day 8 or 9 after the last sporozoite challenge evoked infections in susceptible recipients. These positive transfers were executed 5, 23, 26, and 27 days before development of patent parasitemia in the donor. The five negative transfers were performed at times very similar with respect to onset of patency in the donor, i.e., at 9, 25, 25, 26, and 34 days. Of the 20 subinoculations carried out before the terminal sporozoite challenge, only 1 yielded positive results. The exception, the transfer from A-1690, was performed 62 days before onset of patency.

The data in Table 1, column 4, show that the subinoculees that became infected developed patent parasitemias 7 to 10 days after blood transfer. Prepatent periods such as these are produced by inocula of 10 to 200 trophozoites of the *B* strain (49). Assuming that the volume of blood transferred approximated 1/25th of the total blood volume of the average-sized donor, that subject had a total burden of only 250 to 5,000 infective erythrocytic parasites on the day of maturation of the tissue schizonts, when, as judged by events in untreated controls, flooding of the blood with infective erythrocytic

TABLE 1. Protection accorded by a single 50-mg/kg dose of CGT-P, lot 8652,[a] against repeated challenges with sporozoites of the *B* strain

Monkey no.	Sporozoite challenges[b]		Results of subinoculations on days after dosage with CGT-P[c]	Day of patency after dosage with CGT-P (day after last challenge)[d]
	No.	Days after dosage with CGT-P		
A-1688	7	7, 35, 65, 95, 126, 159, 186	None performed	194 (8)
A-1685	7	7, 35, 65, 95, 126, 159, 186	195_{9}	218 (32)
A-1687	7	7, 35, 65, 95, 126, 159, 186	195_{neg}	220 (34)
A-1683	9	7, 35, 65, 95, 126, 159, 186, 218, 245	195_{neg}, 227_{10}	253 (8)
A-1692	9	7, 35, 65, 95, 126, 159, 186, 218, 245	195_{neg}, 227_{neg}, 253_{neg}	278 (33)
A-1691	10	7, 35, 65, 95, 126, 159, 186, 218, 245, 280	195_{neg}, 227_{neg}, 253_{neg}, 288_{neg}	297 (17)
A-1690	10	7, 35, 65, 95, 126, 159, 186, 218, 245, 280	195_{neg}, 227_{neg}, 253_{9}, 288_{8}	315 (35)
A-1684	11	7, 35, 65, 95, 126, 159, 186, 218, 245, 280, 313	195_{neg}, 227_{neg}, 253_{neg}, 288_{neg}	322 (9)
A-1686	11	7, 35, 65, 95, 126, 159, 186, 218, 245, 280, 313	195_{neg}, 227_{neg}, 253_{neg}, 288_{neg}, 322_{7}	327 (14)
A-1694	11	7, 35, 65, 95, 126, 159, 186, 218, 245, 280, 313	195_{neg}, 227_{neg}, 253_{neg}, 288_{neg}, 322_{neg}	348 (35)
A-1679	3	7, 313, 393	None performed	401 (8)
A-1681	8	7, 313, 393, 420, 455, 483, 517, 537	None performed	562 (25)
A-1854	6	21, 53, 80, 115, 148, 180	None performed	188 (8)
A-1769	6	21, 53, 80, 115, 148, 180	None performed	194 (14)
A-1765	11	21, 53, 80, 115, 148, 180, 208, 236, 267, 290, 318	None performed	329 (11)

[a] Dose of CGT-P expressed as equivalent of CGT; lot 8652 was a suspension of particles 1 to 105 μ in greatest dimensions in benzyl benzoate and castor oil.
[b] The initial inoculum was 1.3×10^6 sporozoites for the first 12 monkeys listed in column 1 and 7.3×10^5 sporozoites for A-1854, A-1769, and A-1765. Subsequent inocula for the 15 monkeys ranged from 1.6×10^5 to 2.5×10^6 sporozoites. There were 30 untreated control monkeys for 28 separate inocula: in 25, parasitemias became patent on day 8 after challenge; in 5, on day 9.
[c] Subinoculations of blood from monkeys dosed with CGT-P to clean, untreated monkeys were carried out 9 days after the 7th and 8th sporozoite challenges, 8 days after the 9th and 10th challenges, and 9 days after the 11th challenge. The result of this transfer is indicated by the subscript, as either negative (neg) or day of onset of patent parasitemia in the subinoculee.
[d] The mean duration of protection, derived from the dates of the last sporozoite challenges listed in column 3, was 277 (±97) days. This is probably a conservative value since in most subjects the days from challenge to patency exceeded the 8- to 9-day interval common to untreated controls.

parasites took place (49). This burden probably represented only a small fraction of the numbers of infective parasites derived from the tissue schizonts, the majority having been eliminated by exposure to CGT continuously released from the CGT-P depot.

(ii) Protection against a single sporozoite challenge. This series of experiments, aimed at defining the protection accorded by a single dose of CGT-P against a single sporozoite challenge, involved work with 162 monkeys: 53 dosed with CGT-P and 22 untreated controls (17 for initial challenge, 5 for rechallenge phases of the series), all challenged with sporozoites, and 87 recipients of blood drawn at various times after sporozoite challenge from 10 of the 53 recipients of the test compound. The dose of CGT-P of the large-sized particle lot was equivalent to 50.0 mg of CGT per kg of body weight.

The inocula in experiments A through D ranged from 1.6×10^5 to 1.4×10^6 sporozoites, each monkey in an individual experiment receiving an inoculum of the same size. Inocula of six different sizes, ranging from 1.8×10^4 to 9.6×10^6 sporozoites, were used in experiment E, which was designed specifically to determine to what extent protection accorded by CGT-P was affected by the size of the inoculum.

Of the 53 monkeys challenged once 7 days after dosage with CGT-P, 35 developed patent parasitemias (Table 2). The mean time between dosage and this event was 247 (standard deviation, ±55) days. Parasitemias of 31 of the 35 subjects became patent between days 198 and 297 after

TABLE 2. Protection accorded by a 50-mg/kg dose of CGT-P, lot 8652,[a] against a single challenge with sporozoites of the *B* strain

Expt	No. of monkeys inoculated[b]	No. of sporozoites in inoculum[c]	Days from dosage to patency in:		
			Individual monkeys[d]	Monkey groups	
				With patent infections[e]	All
A	8	1.3×10^6	>313 for all 8 monkeys	No data	>313
B	3	1.4×10^6	232, 261, 268	254 ± 19	254
C	10	9.1×10^5	118, 223, 237, 242, 244, 256, 278, 287, >403, >403	236 ± 52	>269
D	5	1.6×10^5	238, 250, 276, 277, >405	260 ± 19	>289
E	5	1.8×10^4	259, 294, >485, >499, >499	277	>407
	8	9.6×10^4–1.8×10^5	213, 233, 252, 262, 419, >499, >499, >563	276 ± 82	>368
	9	9.6×10^5–1.8×10^6	198, 227, 241, 247, 248, 254, 297, 314, >499	253 ± 37	>281
	5	9.6×10^6	59, 227, 227, 241, 248	200 ± 80	200

[a] Dose of CGT-P expressed as equivalent of CGT; lot 8652 was a suspension of particles 1 to 105 μ in greatest dimensions in benzyl benzoate and castor oil.
[b] There were five untreated control monkeys for experiments A to D: parasitemias of the four committed to experiments A, B, and C became patent on day 8 after challenge; that of the fifth, committed to experiment D, became patent on day 9. There were 12 untreated control monkeys for experiment E: parasitemias of the two recipients of 1.8×10^4 sporozoites became patent on days 11 and 14; those of the two recipients of 9.6×10^4 sporozoites, on days 8 and 9; and those of the eight recipients of other inocula, on day 8.
[c] All treated monkeys were inoculated 7 days after dosage with CGT-P.
[d] See Table 4 for further information on monkeys who did not develop patent infections during the period of observation in these experiments.
[e] Mean ± standard deviation.

TABLE 3. Results of subinoculations of blood from recipients of a 50-mg/kg dose of CGT-P, lot 8652,[a] carried out at various times during the thick-blood-film-negative period that separated challenge with sporozoites of the *B* strain from onset of patency

Donor[b]		Recipients of blood from donor		
		Days before onset of patency in donor		
Monkey no.	Day of patency after dosage with CGT-P	Negative subinoculees	Positive subinoculees	Days to patency in positive subinoculees
Mmu 146	118	104, 73, 42, 14	None	
Mmu 119	223	209, 178, 147, 119, 87, 57, 25	None	
Mmu 138	237	223, 192, 161, 133, 101, 71, 39, 10	None	
Mmu 149	242	228, 197, 166, 138, 106, 76, 44	13	11
Mmu 137	244	230, 199, 168, 140, 108, 78, 46	15	16
Mmu 113	256	242, 211, 180, 152, 120, 90, 58, 29	None	
Mmu 147	278	264, 233, 202, 174, 142, 112, 80, 51	14	8
Mmu 143	287	273, 242, 211, 183, 151, 121, 89, 60, 29	None	
Mmu 115	>403	389, 358, 327, 299, 267, 237, 205, 176, 145, 114, 84, 54, 23	None	
Mmu 120	>403	389, 358, 327, 299, 267, 237, 205, 176, 145, 114, 84, 54, 23	None	

[a] Dose of CGT-P expressed as equivalent of CGT; lot 8652 was a suspension of particles 1 to 105 μ in greatest dimensions in benzyl benzoate and castor oil.
[b] Donors were challenged with 9.1×10^5 sporozoites of the *B* strain 7 days after dosage with CGT-P.

dosage, and those of the four remaining subjects became patent on days 59, 118, 314, and 419. Of the 53 monkeys challenged, 18 did not develop patent parasitemias during postdosage observation periods ranging from 313 to 563 days. The susceptibilities of 17 of these 18 animals to rechallenge will be related later.

The influence of inoculum size on duration of protection conferred by dosage with CGT-P was evaluated indirectly in experiments A through D and directly in experiment E (Table 2). In the first group of experiments, each with an inoculum derived from a different mosquito source, the time to onset of patency did not appear to be related to inoculum size. In experiment E, with inocula ranging from 1.8×10^4 to 9.6×10^6 sporozoites derived from the same lot of infected mosquitoes, there appeared to be an inverse relation between inoculum size and both time to onset of patency and completeness of protection. This is best shown by comparing the data on the five subjects inoculated with 1.8×10^4 sporozoites with those on the five monkeys challenged with 9.6×10^6 sporozoites. All of the latter group developed patent parasitemias within 248 days of dosage. Only two of the former group exhibited patent parasitemias, these on days 259 and 294 after dosage.

To provide a more critical test of the absence of parasitemia than could be obtained from thick blood film examinations, 10- to 15-ml volumes of blood were drawn repetitively from each of the 10 monkeys in experiment C (Table 2) and transferred to susceptible recipients. These subinoculations were initiated 8 days after sporozoite challenge (the day of patency in the two untreated control monkeys) and were repeated at 28- to 32-day intervals until parasitemias of the donor CGT-P-dosed monkeys were patent. Altogether, 87 subinoculations were performed, 4 to 13 per monkey (Table 3). Of the 87, only 3 were positive. The latter were carried out 13, 14, and 15 days before onset of patency in the donor. The 11- and 16-day intervals between transfer of blood and onset of patency in two subjects are compatible with introduction of, at most, 10 erythrocytic parasites, approximately 1 in each milliliter of blood transferred. It is noteworthy that two other subinoculations carried out 10 and 14 days before onset of patency in donor monkeys (Mmu 138 and Mmu 146) yielded negative results. The overall outcome of this subinoculation study supports the view that the blood of the CGT-P-dosed subject is almost invariably free of parasites during all but the terminal end of the period, when thick blood films are negative.

As indicated in Table 4, column 3, 17 of the 18 monkeys listed in Table 2 who did not exhibit parasitemia for 306 to

TABLE 4. Responses to rechallenge of recipients of CGT-P, lot 8652,[a] refractory to the initial challenge with sporozoites of the *B* strain

Monkey no.[b]	Day of splenectomy after dosage with CGT-P	Rechallenge			
		Day after dosage with CGT-P[c]	Parasitemia		
			Patent (day p.i.)[d]	Peak	
				Day[e]	Height[f]
A-1672	292	313	8 (321)	6	44
A-1674	292	313	8 (321)	6	62
A-1676	292	313	8 (321)	6	33
A-1682	292	313	8 (321)	6	31
A-1671	292	313	11 (324)	7	103
A-1677	292	313	22 (335)	6	56
A-1679	292	313	—[g]	—	—
A-1681	292	313	—	—	—
Mmu 115	ND	403	9 (412)	9	605
Mmu 120	ND	403	9 (412)	13	928
A-2896	ND	405	28 (433)	12	25
Mmu 672	468	499	8 (507)	7	1,736
Mmu 678	468	499	8 (507)	7	738
Mmu 681	468	499	8 (507)	7	4,640
Mmu 692	468	499	8 (507)	16	1,000
Mmu 707	468	499	8 (507)	7	1,808
Mmu 649	535	563	8 (571)	7	2,464

[a] Lot 8652 was a suspension of particles 1 to 105 μ in greatest dimensions in benzyl benzoate and castor oil.
[b] A-1672 through A-1681 were derived from experiment A, Mmu 115 and Mmu 120 from experiment C, A-2896 from experiment D, and Mmu 672 through Mmu 649 from experiment E (Table 2). The rechallenge inocula were: 6.9×10^4 sporozoites for A-1672 through A-1681; 4.1×10^5 sporozoites for Mmu 115 and Mmu 120; 2.9×10^6 sporozoites for A-2896; 3.4×10^6 sporozoites for Mmu 672 through Mmu 707; and 3.4×10^6 sporozoites for Mmu 649. There were five control monkeys for these inocula; each developed a patent parasitemia on day 8 after challenge.
[c] The initial challenge was performed on day 7 after dosage with CGT-P; thus, 306 to 556 days separated the initial challenge from the rechallenge.
[d] Numbers in parentheses refer to day of patency after dosage with CGT-P. p.i., Postinoculation.
[e] Day after onset of patency.
[f] Number of parasites per 10^4 erythrocytes.
[g] —, Not patent through day 393.

TABLE 5. Capacity of a 50-mg/kg dose of CGT-P, lot 8652,[a] to control parasitemia and protect against relapses in monkeys infected with sporozoites of the *B* strain

Monkey no.[b]	No. of parasites per 10^4 RBC on day of dosage with CGT-P[c]	Response to dosage with CGT-P: Days to parasite clearance[d]		Days from dosage with CGT-P to relapse	
		Trophozoites and gametocytes	Drug forms	Individual[e]	Mean ± SD
A-2928	321	4	7	201	
A-2642	92	4	7	202	
A-2913	94	4	7	211	
A-2611	164	4	7	215	
A-2890	444	4	7	217	
A-2916	444	4	7	239	
A-2895	246	3	7	251	288 ± 114
A-2670	176	4	7	259	
A-2892	315	3	7	262	
A-2707	134	3	7	297	
A-2933	312	5	8	335	
A-2584	266	3	7	521	
A-2662	270	4	7	537	

[a] Dose of CGT-P expressed as equivalent of CGT; lot 8652 was a suspension of particles 1 to 105 μ in greatest dimensions in benzyl benzoate and castor oil.

[b] All monkeys were inoculated with 8.6×10^4 sporozoites; parasitemia of A-2584 was patent on day 8 after inoculation; parasitemias of other monkeys, including one untreated control, were patent on day 9.

[c] CGT-P was administered on day 15 after inoculation. RBC, Erythrocytes.

[d] Measured by failure to detect normal or abnormal (or both) parasite forms during 20-min search of thick blood films.

[e] These relapses occurred 107 to 449 days after splenectomies performed 88 to 94 days after dosage with CGT-P.

556 days after the primary sporozoite challenge were rechallenged 313 to 563 days after dosage with CGT-P. Of the 18, 15 had been splenectomized 292 to 535 days after dosage with CGT-P. One of this group died of shigellosis (*Shigella flexneri* type 4) 17 days after surgery. The remaining 14 were rechallenged 21 to 31 days after splenectomy. Five untreated monkeys, all with intact spleens, served as controls for the above rechallenges. Parasitemias of these five subjects became patent 8 days after sporozoite inoculation and peaked 6 to 8 days later at levels of 650 to 900 parasites per 10^4 erythrocytes.

Of the eight monkeys rechallenged 313 days after dosage with CGT-P, two did not become infected during the 80-day postinoculation observation period. The intervals between sporozoite rechallenge and onset of patent parasitemias were 8 days for four of the six subjects that became infected and 11 and 22 days for the remaining two. Parasitemias of all six monkeys peaked 6 to 7 days after onset of patency at levels of 31 to 103 parasites per 10^4 erythrocytes, low not only for intact monkeys challenged with the *B* strain (49), but also extremely low for splenectomized subjects (L. H. Schmidt, unpublished observations). Of the nine subjects rechallenged 403 to 563 days after dosage with CGT-P, only one, A-2896, displayed either a delay in establishment of a patent parasitemia (20 days in this case) or a peak parasitemia lower than normal. The peaks of 1,000 to 4,640 parasites per 10^4 erythrocytes encountered on rechallenge of five of the six splenectomized monkeys are characteristic of the primary peaks encountered in untreated infections with the *B* strain in such subjects (L. H. Schmidt, unpublished observations). These observations indicate that in some cases there is an output of CGT from the CGT-P tissue depot 313 days after dosage which suffices to either prevent or retard parasite multiplication, but that only rarely is there an effective output 400 days after CGT-P dosage. This conclusion, based on the duration of protection attained in these single-challenge experiments, is compatible with the results of the multiple-challenge experiments described previously.

(iii) Activity of CGT-P against established sporozoite-induced infections. Since in field use a repository antimalarial agent would be administered to some individuals with active infections, as well as to persons with quiescent infections and those not infected at all, it seemed important to determine how CGT-P would perform in subjects with active disease. Fourteen monkeys, each inoculated with 8.6×10^4 sporozoites, were used in this appraisal. Parasitemia was established in 1 monkey on day 8 after sporozoite challenge and in the other 13 monkeys on day 9. Thirteen subjects received a single dose of the large-sized particle preparation of CGT-P equivalent to 50.0 mg of CGT per kg on day 15 after sporozoite inoculation, during the ascending phase of the primary attack.

Dosage with CGT-P effected clearance of normal asexual and sexual forms of *P. cynomolgi* in 3 to 5 days and elimination of all morphologically atypical parasites (drug forms) in 7 to 8 days (Table 5). As measured by results of repetitive examinations of thick films, the blood remained free of parasites for 201 to 537 days after CGT-P dosage. It should be noted that the monkeys in this study were splenectomized 88 to 94 days after dosage in an unsuccessful effort to hasten relapse. The mean interval between dosage and relapse, 288 days, was not materially different from the mean duration of protection accorded when subjects were challenged with sporozoites 7 days after dosage (Table 2).

(iv) Influence of dose, suspending medium, and, indirectly, particle size on protection accorded by CGT-P against repeated challenges with sporozoites. The primary purposes of this study were to evaluate the influences of size of dose and character of the suspending medium on the activity of CGT-P. The use in this experiment of a bulk preparation of CGT-P made up of 10- to 40-μ particles, rather than the formulated 1- to 105-μ particle preparation used in previous experiments, added a fortuitous and uncontrolled assessment of the influence of particle size on activity to the primary objectives. Altogether, 66 monkeys were used in the study. Of this number, 50 were recipients of single doses of CGT-P equivalent to 3.125 to 50.0 mg of CGT per kg: five recipients of each of these doses in the aqueous vehicle, five in the oleaginous vehicle. Each of the 50 monkeys was challenged with sporozoites 7 days after dosage with CGT-P and at 20- to 50-day intervals thereafter until all had developed patent parasitemias. The remaining 16 subjects were untreated controls for the total of 13 sporozoite challenges.

The results of this experiment (Table 6) show that the protection accorded by CGT-P was related directly to the size of dose of this agent and that the composition of the suspending medium had little effect on duration of protection attained. Animal-to-animal variations in results were relatively small except at the 50.0-mg/kg dose, where they were substantial, with protection ranging from 99 to 410 days and from 84 to 327 days in recipients of the agent in the aqueous and oleaginous vehicles, respectively.

Comparison of all results attained with a 50.0-mg/kg dose in this study with those attained with this dose in the oleaginous vehicle (Table 1) indicates that the protection accorded by medium-sized particle preparations of CGT-P is substantially less and somewhat more variable than the protection attained with the large-sized particle preparation. Thus, the mean interval between dosage and onset of parasitemia was 167 days (standard deviation, ±111) for

recipients of the medium-sized particle lot in aqueous or oleaginous vehicle and 296 days (standard deviation, ±98) for recipients of the large-sized particle lot in the oleaginous vehicle.

(v) Susceptibility to CGT hydrochloride of blood schizonts that developed in recipients of CGT-P. This study was aimed at determining whether erythrocytic parasites that emerged in recipients of CGT-P at the end of the period of protection or in subinoculees of recipients of CGT-P were normally susceptible or resistant to CGT. It involved work with 112 monkeys, 39 infected with trophozoites and 73 with sporozoites. The latter included 49 recipients of the medium-sized particle lot of CGT-P (Table 6), with breakthroughs 23 to 327 days after dosage, and 24 recipients of the large-sized particle lot (Tables 1 and 2), with breakthroughs 198 to 562 days after dosage. Of the 39 monkeys infected with trophozoites, 12 were subinoculees of recipients of CGT-P and 27 were infected with parasites of the passage *B* strain. Of these 27, 2 served as untreated controls, 25 to provide data on responses to CGT-hydrochloride of infections with the stock strain against which the responses to this agent of infections with subjects exposed to CGT-P could be weighed. Infections were routinely treated with CGT hydrochloride during the ascending phase of the primary attack, when parasitemias involved between 10 and 100 parasites per 10^4 erythrocytes.

The water-soluble salt was administered via stomach tube, in doses equivalent to 0.075, 0.3, or 0.6 mg of CGT per kg, once daily for 7 consecutive days. Selection of these doses was based on the results of previous evaluations of the activity of CGT hydrochloride against infections with the *M* strain of *P. cynomolgi* (55). Thick and thin blood films were prepared, stained, and studied daily from the onset of parasitemia to confirmation of relapse in infections with sporozoites or to recrudescence or cure (49) in infections with trophozoites.

The results of this study (Table 7) indicate that the exposure to CGT that took place in recipients of CGT-P did not induce emergence of CGT-resistant parasites. Infections in subinoculees of recipients of CGT-P responded to treatment with 0.3-mg/kg doses of CGT hydrochloride almost precisely as did infections in subjects inoculated with trophozoites of the stock *B* strain. This regimen effected clearance of parasitemia in 7.3 and 6.2 days and cured 75 and 80% of the monkeys in the respective groups. Daily doses of either 0.3 or 0.6 mg/kg effected clearance of parasitemia in essen-

TABLE 6. Influences of dose and suspending vehicle on protection accorded by CGT-P, lot 4699-X74,[a] against repeated challenges with sporozoites of the *B* strain

Dose of CGT-P (mg/kg)	Suspending vehicle[b]	No. of monkeys	Sporozoite challenges[c]		Days from dosage to patency	
			No.	Days after dosage with CGT-P	Individual monkeys	Group mean ± SD
3.125	A	4	1	7	24, 31, 37, 44	36.8 ± 9.7
		1	2	7, 38	48	
	O	5	1	7	23, 32, 34, 35, 36	32.0 ± 5.2
6.25	A	2	1	7	31, 38	43.4 ± 9.0
		3	2	7, 38	47, 47, 54	
	O	5	1	7	30, 37, 40, 43, 45	39.0 ± 5.9
12.5	A	5	2	7, 38	47, 50, 50, 59, 64	54.0 ± 7.2
	O	5	2	7, 38	48, 55, 62, 71, 72	61.6 ± 10.3
25.0	A	1	2	7, 38	70	94.2 ± 18.0
		3	3	7, 38, 71	86, 94, 103	
		1	4	7, 38, 71, 103	118	
	O	4	2	7, 38	53, 60, 71, 73	72.0 ± 19.2
		1	3	7, 38, 71	103	
50.0	A	2	3	7, 38, 71	99, 110	186 ± 129
		1	4	7, 38, 71, 103	131	
		1	6	7, 38, 71, 103, 131, 159	182	
		1	13	7, 38, 71, 103, 131, 159, 190, 213, 241, 275, 295, 344, 394	410	
	O	3	3	7, 38, 71	84, 95, 106	147 ± 102
		1	4	7, 38, 71, 103	123	
		1	11	7, 38, 71, 103, 131, 159, 190, 213, 241, 275, 295	327	

[a] Dose of CGT-P expressed as equivalent of CGT; lot 4699-X74, from which both aqueous and oleaginous suspensions were prepared, was made up of particles with a mean size of 30 μ (range, 10 to 40 μ).

[b] A, Aqueous preparation, with compound suspended in special vehicle provided by the late Paul Thompson, Research Division, Park, Davis & Co.; O, oleaginous preparation, with compound suspended in benzyl benzoate and castor oil.

[c] For the initial challenge, the inoculum was 5.8×10^5 sporozoites; for the second and third challenges, inocula were 1.6×10^5 and 7.7×10^4 sporozoites, respectively; for the remaining challenges, inocula ranged from 3.1×10^5 to 9.8×10^5 sporozoites. There were 16 untreated control monkeys for the 13 inocula used for as many challenges: in 11, parasitemias became patent on day 8 after challenge; in 5, on day 9.

TABLE 7. Comparative activity of CGT hydrochloride against the blood schizonts of the regular passage line of the *B* strain and schizonts that developed in recipients of CGT-P subsequent to their challenge with sporozoites of the *B* strain

Type of infection[a]	Origins of treated group[b]	Treatment with CGT hydrochloride and response thereto					
		Daily dose (mg/kg)[c]	No. of infections treated			Mean ± SD days from:	
			Total	Cured	Relapsed or recrudesced	First dose to clearance of parasitemia	Last dose to relapse or recrudescence
T	Passage *B* strain (no drug exposure)[d]	0.075	5[e]	0	1	9	8
		0.3	20	16	4	6.2 ± 1.0	9.5 ± 1.9
S	Recipients of CGT-P (lot 4699-X74) that developed patent parasitemias 23–327 days (mean ± SD, 70 ± 50) after dosage	0.3	49	0	49	6.0 ± 1.2	12.3 ± 3.9
S	Recipients of CGT-P (lot 8652) that developed patent parasitemias 220–562 days (mean ± SD, 345 ± 102) after dosage	0.3	8	0	8	7.4 ± 0.9	15.0 ± 6.0
T	Subinoculees from monkeys in Table 1 at either onset of overt patency or just prior thereto	0.3	12	9	3	7.3 ± 1.4	10.0 ± 2.7
S	Recipients of CGT-P (lot 8652) that developed patent parasitemias 198–419 days (mean ± SD, 262 ± 52) after dosage	0.6	16	0	16	7.3 ± 1.4	14.3 ± 5.2

[a] T, Trophozoite (blood) induced; S, sporozoite (mosquito) induced.
[b] Lot 8652 was a suspension of particles 1 to 105 μ in greatest dimensions in benzyl benzoate and castor oil; lot 4699-X74 was a suspension of particles 10 to 40 μ in size in either a proprietary aqueous vehicle or benzyl benzoate and castor oil.
[c] Doses, expressed as equivalent of CGT, were administered once daily for 7 consecutive days.
[d] There were two untreated controls for this group of 25 treated monkeys. Infections in these controls followed a course characteristic of untreated infections with the *B* strain (49).
[e] This dose did not effect clearance of parasitemias in four of the five treated monkeys.

tially this same time interval (6.0 to 7.4 days) in the recipients of CGT-P that had developed infections subsequent to sporozoite challenge. Since neither chlorguanide nor CGT is active against the tissue forms of *P. cynomolgi* (51, 55), clearance of parasitemia was followed by relapse in every case.

Some relationships of the activities of CGT-P to the urinary output of CGT. Earlier studies from our laboratories showed that, when CGT hydrochloride was administered intravenously to normal rhesus monkeys, 86 to 92% of the dose could be recovered within 48 h from the urine as p-chloroaniline-containing equivalents (57). The parent compound, CGT, accounted for 85 to 95% of these equivalents and p-chlorophenylbiguanide accounted for the remainder (57). This demonstration underpinned a number of experiments concerned with the elimination of CGT-P from the site of injection or deposition, two of which will be described here. One of these was aimed at determining the effects of dose and particle size on the time over which measurable quantities of CGT were released from the depot of the pamoic acid salt in the thigh muscle mass. The second was directed toward determining (i) whether the duration of protection associated with delivery of CGT-P extended beyond release of this agent from a depot and (ii) the dimension of the daily output of CGT in urine at onset of patency.

(i) Impacts of CGT-P dose and particle size on output of CGT in urine. This study involved work with 27 female monkeys. Of these subjects, 18, in six subgroups of 3 each, received the medium-sized particle lot 9163 of CGT-P, suspended in the castor oil-benzyl benzoate vehicle, in doses equivalent to 3.125, 6.25, 12.5, 25.0, 50.0, or 100.0 mg of CGT per kg of body weight. Each of six monkeys in a seventh subgroup received the large-sized particle lot 8652 of CGT-P, in the same vehicle, in a dose equivalent to 50.0 mg of CGT per kg of body weight. Each of three monkeys in an eighth subgroup received the vehicle only, at a dose of 0.5 ml/kg of body weight.

Metabolism cages (ca. 40.6 by 40.6 by 61 cm), designed to facilitate quantitative collections of urine (free of feces) in 1,000-ml Florence flasks enclosed in an ice bath, were used in this experiment and the one to follow. Monkeys committed to the study, including the vehicle-treated controls, were placed in these cages on alternate days, starting 14 days before dosage (or the temporal equivalent thereof, in the case of untreated controls) and continuing after dosage until the output of CGT in urine of recipients of CGT-P had fallen to levels that could not be distinguished with certainty from the blank levels of pretreatment control collections. The animals were maintained in these cages without restraint, with access to their regular diet twice daily and unlimited access to drinking water.

The procedure used to measure the output of CGT in urine was a modification of a method developed in our laboratories several years earlier to facilitate studies on the metabolic conversion of various biguanides to CGT (54). This method was in turn a modification of a procedure developed to measure the concentrations of chlorguanide in body fluids and tissues (58, 59). Basically, it involved alkalinization of a fraction of the day's collection of urine, extraction of the CGT therein into an 80:20 (vol/vol) mixture of ethylene dichloride-ethylhexanol, reextraction of the CGT from this solvent mixture into a small volume of 0.25 N HCl, hydroly-

sis of the CGT in the acid extract to p-chloroaniline, and measurement of the latter by the Bratton-Marshall procedure (3). Specifically, 5-ml samples of urine (or urine diluted 1:100 or 1:10 with distilled water when necessary), 1 ml of 10 N NaOH, and 25 ml of ethylene dichloride-ethylhexanol were added in sequence to a 50-ml round-bottomed centrifuge tube fitted with a Teflon-lined screw cap. The tube and contents were shaken vigorously for 30 min and then centrifuged for 15 min at 1,200 × *g*. The aqueous supernatant was removed by aspiration, and 20 ml of the organic phase was transferred to a clean centrifuge tube containing 5 ml of 0.25 N HCl. After being shaken for 15 min and centrifuged for 10 min, approximately 4.5 ml of the acid extract was transferred to an ampoule which was sealed and placed in a 116°C oven for 16 h. After cooling, 2-ml samples were transferred to a microcuvette and reacted ad seriatim by the method of Bratton and Marshall (3) with dilute sodium nitrite, ammonium sulfamate, and N^1-naphthylethylenediamine dihydrochloride. The intensity of the resulting colored solution was measured in a Klett photoelectric colorimeter equipped with a 540 filter. For determination of CGT content of the solution, the colorimeter reading was referred to a curve prepared from reacting various concentrations of p-chloroaniline with the Bratton-Marshall reagents. Results of analyses of duplicate samples from the same hydrolysate varied by less than 2%, and those from hydrolysates of duplicate extracts varied by no more than 5%. Recovery of CGT added to control urine in concentrations ranging from 0.2 to 20.0 μg/ml was 96 ± 3% (mean ± standard deviation). The blank values for urine collected pretreatment from recipients of CGT-P and throughout the observation period on recipients of the vehicle alone ranged from the equivalent of 0.02 to 0.07 μg of CGT per ml, amounting to the equivalent of 4.8 to 16.8 μg of CGT per daily output of urine. The measured outputs of CGT after dosage with CGT-P were corrected for these pretreatment blanks.

As noted previously, urine samples were collected on alternate days. This practice was dictated in part by the availability of metabolism cages and in part by our unwillingness to confine monkeys in small cages for months on end. Since the total output of CGT-P was of concern, it was necessary to estimate the amount excreted on those alternate days when monkeys were not caged. This output was calculated as the mean between amounts recovered on adjacent collection days. This approximation was probably close to actual output, since with few exceptions, the day-to-day elimination of CGT in any one subject followed a regular pattern.

The results of this study showed that both the early and sustained outputs of CGT in urine were affected significantly by particle size (Table 8). There was a veritable outpouring of CGT during the 5 days after dosage with medium-sized particles, amounting to 62% of the 3.125-mg/kg dose, 37% of the 12.5-mg/kg dose, and 16% of the 100.0-mg/kg dose (Table 8, column 4). Nothing comparable to this outpouring occurred after dosage with the large-sized particle preparation. Thus, in the 5 days after administration of a 50.0-mg/kg dose of this preparation, 7% of the total dose was eliminated in the urine, slightly more than one-fourth the output after delivery of the same dose of the medium-sized particle preparation. The impact of particle size on the output of CGT in urine extended well beyond the post-5-day dosage interval. The slope of the elimination curve in this latter period in recipients of the medium-sized preparation was far steeper than that in recipients of the large-sized particle preparation. As a result, the times over which the output of CGT could be measured were substantially shorter in recipients of the medium-sized particles than in those of the large-sized particles, 79 days at the 50.0-mg/kg dose of the former preparation and 184 days at the same dose of the latter (Table 8, column 5).

Assessment of the impacts of dose size on duration of output of CGT was limited to the medium-sized particle preparation. The results of this appraisal showed that the times over which measurable amounts of CGT were eliminated in the urine were directly related to dose size ranging from a mean of 11 days at 3.125 mg/kg to 138 days at 100.0 mg/kg (Table 8, column 5). Although the pattern of these elimination data mirrored that of the therapeutic response data (Table 6), the lengths of time over which CGT could be recovered in urine were always significantly shorter than the duration of protection, reflecting the limits of sensitivity of the analytical procedure.

The day-to-day outputs of CGT in the urine of each of the three recipients of the medium-sized particle preparation of CGT-P in doses equivalent to 50.0 and 100.0 mg of CGT per kg and the six recipients of the large-sized particle preparation in doses of 50.0 mg/kg have been charted in Fig. 1. These charts depict the immediate postdose differences in amounts of CGT and fraction of dose eliminated by recipi-

TABLE 8. Influence of dose and particle size of CGT-P on the early and sustained elimination of CGT in urine and the time over which measurable amounts could be recovered

CGT-P lot no.[a]	Dose[b] (mg/kg)	No. of monkeys	Recovery of CGT-P as CGT equivalents		
			During days 0–5 (% Dose, mean ± SD)	During total observation period	
				Days present (mean ± SD)[c]	% Dose (mean ± SD)
9163	3.125	3	62 ± 4	11 ± 5	69 ± 15
	6.25	3	46 ± 6	19 ± 14	62 ± 7
	12.5	3	37 ± 6	27 ± 3	66 ± 7
	25.0	3	31 ± 8	43 ± 16	72 ± 8
	50.0	3	27 ± 13	79 ± 10	67 ± 8
	100.0	3	16 ± 2	138 ± 4	71 ± 2
8652	50.0	6	7 ± 2	184 ± 27	69 ± 5

[a] Lot 9163 was characterized as a medium-sized particle preparation: 26% of the particles were <23 μ, 54% were 23 to 34 μ, and 20% were >34 μ in greatest dimensions (average, 25 μ). Lot 8652 was characterized as a large-sized particle preparation, with 70% of the particles in excess of 50 μ in greatest dimensions.

[b] Dose is expressed as equivalent of milligrams of CGT per kilogram of body weight.

[c] In amounts exceeding the mean blank values measured in the regularly scheduled collections of urine from three recipients of the vehicle alone and in the predosage collections from each of the 24 recipients of CGT-P.

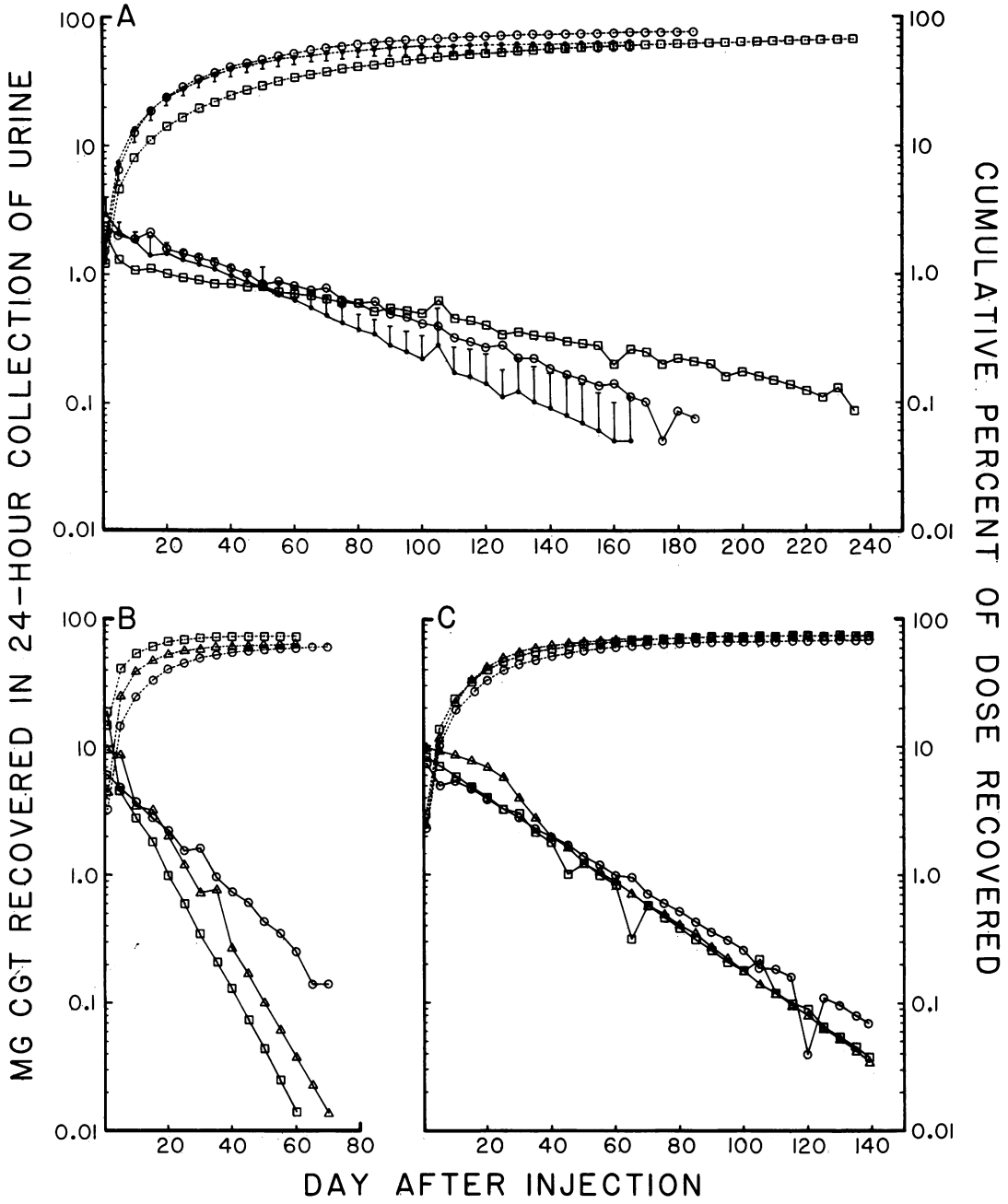

FIG. 1. Influence of size of CGT-P particles on elimination of CGT in urine. ———, Milligrams of CGT recovered; – – –, cumulative percentage of CGT-P dose recovered. (A) Recipients of 50-mg/kg doses of large-sized CGT-P particles. Symbols: ●, mean data ± standard deviation for Mmu 12, 19, 28, and 29; ○, Mmu 38; □, Mmu 48. (B) Recipients of 50-mg/kg doses of medium-sized CGT-P particles. Symbols: △, Mmu 149; ○, Mmu 336; □, Mmu 439. (C) Recipients of 100-mg/kg doses of medium-sized particles. Symbols: △, Mmu 113; ○, Mmu 137; □, Mmu 444.

ents of the medium- and large-sized particle preparations, the assumption of regular elimination patterns by all subjects after this initial flooding, the monkey-to-monkey variations in these patterns, the differences in the slopes of the elimination curves in recipients of the various-sized particle preparations, and, in line with the latter, the time period over which measurable amounts of CGT were excreted. The charts also show that the total cumulative output of CGT was still increasing, although in exceedingly small increments, at the time when daily elimination could no longer be measured accurately.

(ii) Release of CGT from intraabdominal implants of CGT-P as related to duration of protection against sporozoite challenge. This experiment was served by 14 female monkeys, 9 of which received intraabdominal implants of CGT-P equivalent to 100.0 mg of CGT per kg, 3 of which received an intramuscular dose of CGT-P equivalent to 50.0 mg of CGT per kg, and 2 of which received an intramuscular dose of 0.5

ml of the castor oil-benzyl benzoate vehicle per kg. Lot 8652, the ampouled, large-sized particle preparation of CGT-P, was used for both implantation and injection. The implant vessel consisted of a 40-mm length of 3-mm-diameter sterile Visking tubing (average pore radius, 2.4 nm) partially filled with the requisite amount of the CGT-P suspension and tied with nylon thread at both ends, which were then inverted. The prepared tube was placed in the abdominal cavity between the dome of the right lobe of the liver and the right leaf of the diaphragm. Placement was effected via an incision through the skin, underlying muscle fascia, and upper right abdominal wall and was carried out under sodium pentobarbital anesthesia, with strict attention to asepsis.

The 14 implantations and intramuscular dosages with CGT-P or vehicle were carried out on the same day. Seven days later, each monkey was inoculated intravenously with 1.5×10^6 sporozoites. Preparation of thick and thin blood films for parasitological study was initiated 7 days after sporozoite challenge and repeated daily on all animals until observations on those with intraabdominal implants were completed. From that time to day 279 after sporozoite challenge, the recipients of intramuscularly administered CGT-P and untreated controls were subjected to blood film examinations on a Monday-Wednesday-Friday or Tuesday-Thursday-Saturday schedule.

Via laparotomies, performed under sodium pentobarbital anesthesia, the implants from two groups, each containing three monkeys, were removed 22 and 43 days after implantation (15 and 36 days after sporozoite challenge) and from two monkeys of a third group 72 days after implantation (65 days after challenge). The implant from the third monkey in the latter group could not be located at laparotomy on day 72 and was never recovered.

Urine was collected over periods of 24 h on alternate days, from 14 days before implantation or dosage to 5 days after removal of implants or 121 days after dosage in the other subjects. Collections were terminated on day 121, when the output of CGT by the monkey with the residual implant reached essentially pretreatment blank levels. The amount of CGT in each day's collection was determined as described in the preceding section, with correction of output for pretreatment blanks.

The results of the parasitological component of this experiment show that parasitemias of the recipients of the vehicle only (the untreated controls) became patent 8 and 9 days after sporozoite challenge and followed courses (Fig. 2A) during the ensuing 270- to 271-day post-patency observation period characteristic of untreated infections with sporozoites of the *B* strain (49). Recipients of implants of CGT-P exhibited no evidence of parasitemia until the implants were removed (Fig. 2C–E). Irrespective of the time of removal, parasitemias became patent 7 to 8 days later and, monkey 169 excepted, peaked at levels and times not different from those in the untreated controls. The parasitemia of monkey 169 peaked at a normal time, but at a below-normal level (136 parasites per 10^4 erythrocytes). The parasitemia of monkey 152, the monkey from whom the implant could not be recovered, became patent 128 days after implantation and evolved normally in all respects. Parasitemias of the three recipients of intramuscularly administered CGT-P became patent 231, 261, and 268 days after dosage and progressed normally (Fig. 2B). The duration of protection for these subjects was approximately twice that attained in monkey 152, who received twice the dose of CGT-P via implant.

Daily outputs of CGT by groups of recipients of implanted or injected CGT-P have been summarized in Fig. 3. Outputs by the nine recipients of implants followed a remarkably similar pattern for the first 20 days after dosage, averaging 1 mg of CGT per day immediately after implantation and declining to approximately 0.15 mg by day 20 (Fig. 3B–D). Average outputs were sustained at the latter level or slightly higher for the remainder of the implant periods by the groups of monkeys whose implants were removed 43 and 72 days after dosage (Fig. 3B and D). The output of CGT by each of the eight subjects from whom the Visking tubes were recovered fell below measurable levels within 3 to 4 days of removal of the implant (Fig. 3B–D). The output by monkey 152, the ninth member of the implant groups, declined slowly but steadily, from 0.39 mg on day 71 after implantation (just before the unsuccessful attempt to recover the implant) to 0.04 mg on day 121, 7 days before onset of patency (Fig. 3B). The daily output of CGT by recipients of intramuscular doses of CGT-P approximated 3 mg 10 days after dosage, substantially greater than the output by recipients of implants at that time, and declined steadily thereafter, reaching 0.15 mg 121 days after dosage (Fig. 3A). Had this rate of decline continued, a daily output of 0.03 mg would have been reached around posttreatment day 250, approximately the time when parasitemias became patent.

The data provided by this experiment have at least two noteworthy implications. First, the onset of patency within no more than 5 to 7 days after clearance of measurable amounts of CGT from the urine indicates that either this agent is without effect on development and persistence of the tissue stages or, if it has such effects, they are rapidly and fully reversible. Second, the relatively prompt onset of parasitemia that occurred when the daily output of CGT in urine fell below 0.04 mg indicates that this level is critical for protection. Results of previous studies on the urinary elimination of CGT after intravenous administration of CGT hydrochloride (57) suggest that it would correspond to release of 0.045 mg of CGT from the tissue depot. This amount would be the equivalent of a dose of 0.015 mg/kg (0.18 mg/m^2) for monkeys of the size used in these studies, approximately 1/40th of the single intravenous dose of CGT required for elimination of parasitemia in 90% of subjects with established infections. This activity differential testifies to the benefits derived from slow, steady release of CGT.

Activities of CGT-P as affected by pyrimethamine resistance. Pyrimethamine-resistant strains of *P. falciparum* are distributed worldwide, wherever infections with this plasmodium are transmitted (66). In some settings, but reportedly not all, infections with these resistant strains are cross-resistant to chlorguanide (66) and presumably to its active metabolite, CGT (6, 7, 18, 30). Such cross-resistance could seriously impair the effectiveness of CGT-P as a repository antimalarial agent. This made it imperative to evaluate the impact of pyrimethamine resistance on the activity of CGT-P. The series of studies that follow were directed to that assessment.

(i) Comparative efficacies against challenges with sporozoites of the pyrimethamine-susceptible *Ro* and pyrimethamine-resistant *Ro/PM* strains. This issue was pursued in two parallel experiments with the *Ro* and *Ro/PM* strains and four additional experiments with the latter strain only. In all, they involved work on 57 monkeys, 10 inoculated with sporozoites of the *Ro* strain and 47 with sporozoites of the *Ro/PM* strain. Seven of the monkeys inoculated with sporozoites of the *Ro* strain and 37 inoculated with sporozoites of the *Ro/PM* strain were given a dose of CGT-P equivalent to 50.0 mg of CGT per kg 7 days before sporozoite challenge. The

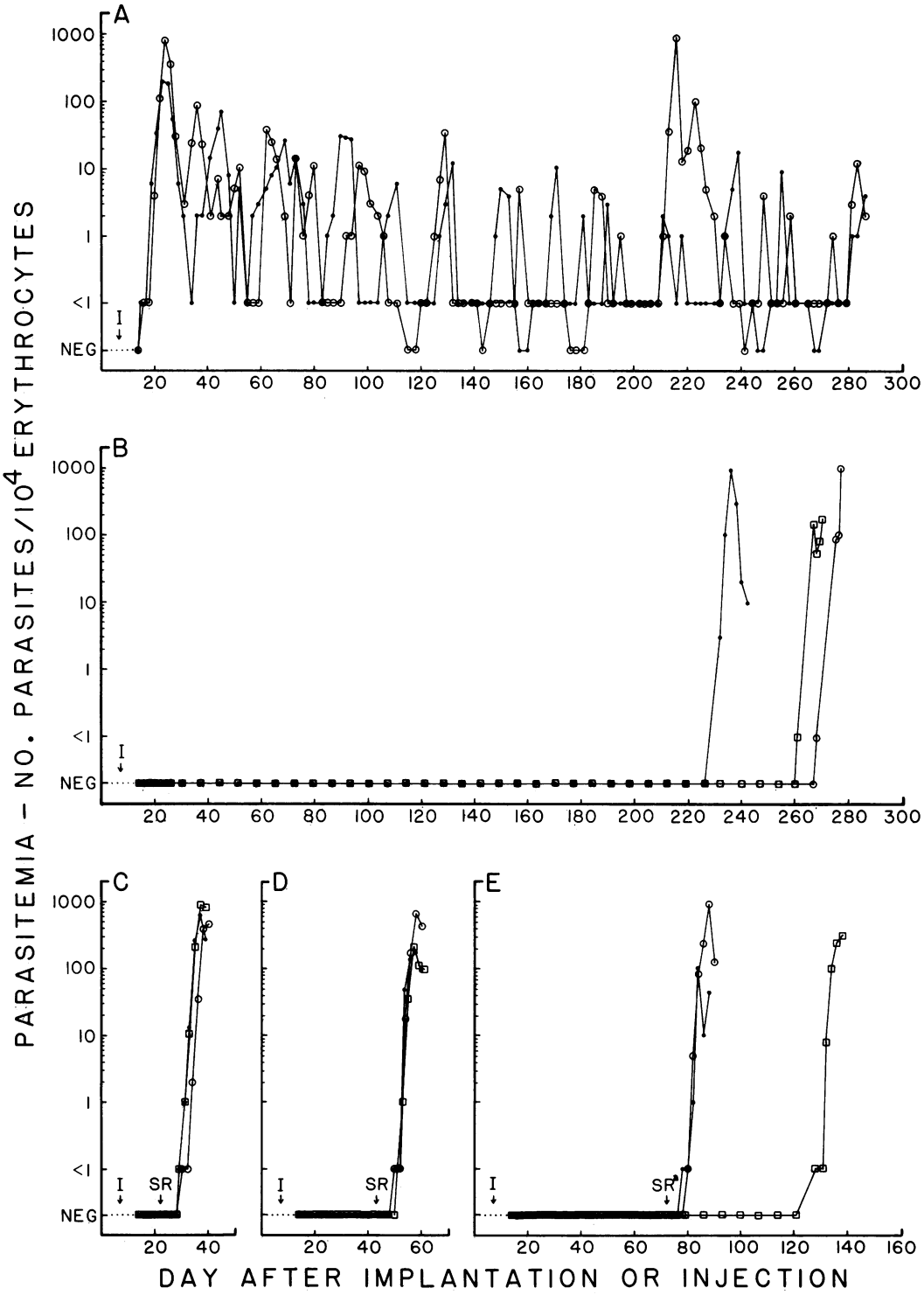

FIG. 2. Duration of protection against overt infections accorded by removable intraabdominal implants of CGT-P as compared with that provided by intramuscular doses. I, Inoculation with sporozoites; SR, sac removed. (A) Recipients of vehicle only. Symbols: ●, Mmu 223; ○, Mmu 224. (B) Recipients of CGT-P intramuscularly. Symbols: ●, Mmu 154; ○, Mmu 155; □, Mmu 163. (C–E) Recipients of implants of CGT-P. (C) Implants removed on day 15 after inoculation. Symbols: ●, Mmu 157; ○, Mmu 175; □, Mmu 184. (D) Implants removed on day 36 after inoculation. Symbols: ●, Mmu 124; ○, Mmu 183; □, Mmu 188. (E) Implants removed on day 65 after inoculation. Symbols: ●, Mmu 169; ○, Mmu 171. Implant not recovered on day 65. □, Mmu 152.

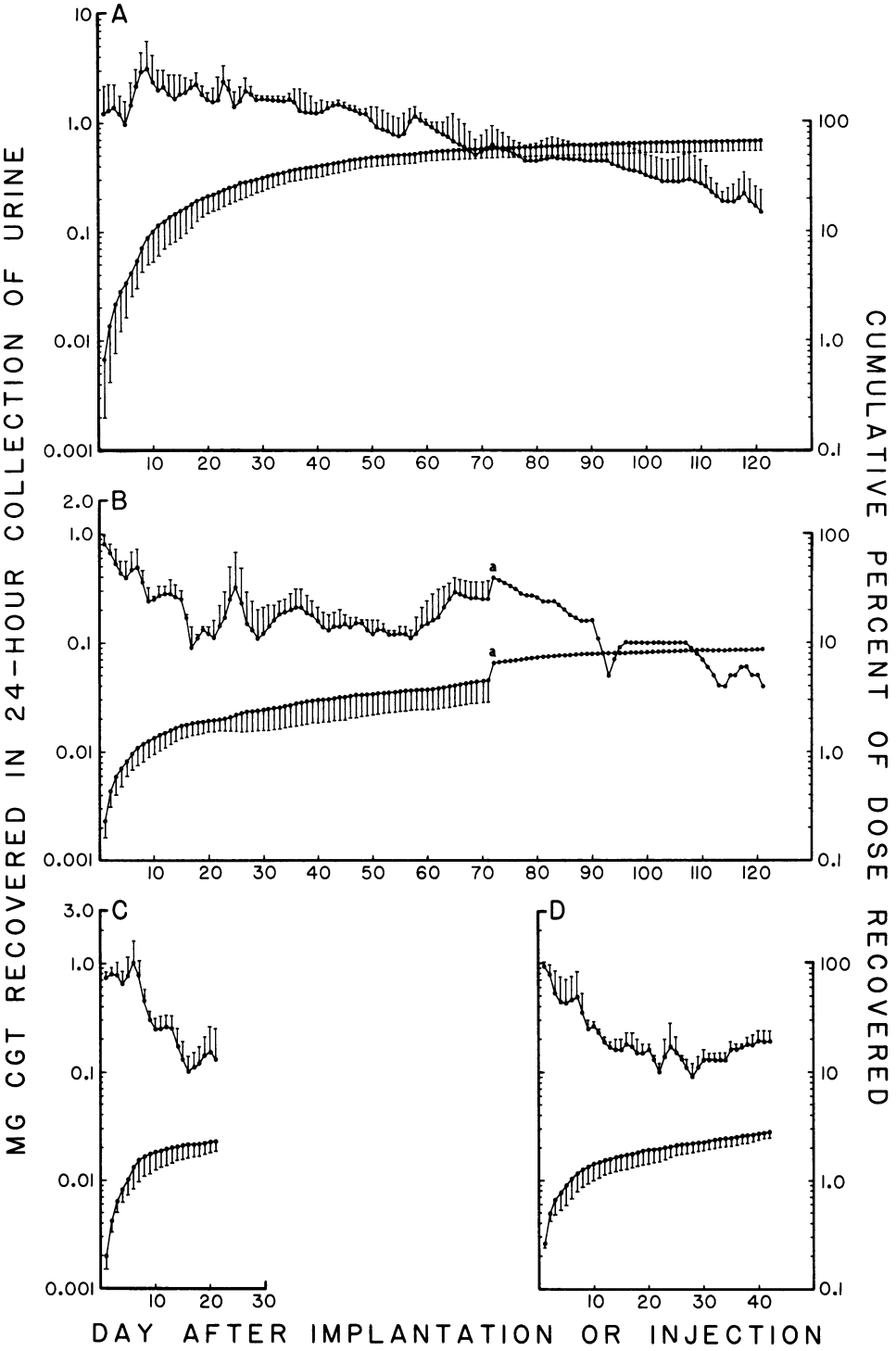

FIG. 3. Elimination of CGT in urine of recipients of CGT-P in removable intraabdominal implants as compared with that in recipients of intramuscular doses. ⊥—⊥, Milligrams of CGT recovered ± standard deviation; ⊥—⊥, cumulative percentage of CGT-P dose recovered. (A) Recipients of CGT-P intramuscularly; composite data on Mmu 154, 155, and 163. (B–D) Recipients of implants of CGT-P. (B) Composite data on Mmu 169, 171, and 152 to day 72 after implantation, when implants of Mmu 169 and 171 were removed; from "a" on, data on Mmu 152 from whom implant could not be recovered. (C) Composite data on Mmu 157, 175, and 184 to day 22 after implantation, when implants were removed. (D) Composite data on Mmu 124, 183, and 188 to day 43 after implantation, when implants were removed.

TABLE 9. Protection accorded by a 50-mg/kg dose of CGT-P against a single challenge with sporozoites of the *Ro* or *Ro/PM* strain

Strain	Expt[a]	No. of sporozoites in inoculum[b]	No. of monkeys inoculated	Days from dosage to patency[c]	
				Individual monkeys	Mean ± SD
Ro	A	2.0×10^6	4	>352, >352, >352, >352	>352[d]
	B	1.8×10^6	3	116, 578[e], >829[e]	>508
Ro/PM	A	1.3×10^6	5	26, 33, 38, 54, 89	48 ± 25
	B	6.1×10^5	9	16, 22, 26, 35, 35, 43, 47, 68, 86	42 ± 23
	C	5.1×10^5	10	30, 34, 39, 40, 42, 42, 44, 46, 49, 70	44 ± 11
	D	9.1×10^4	5	21, 23, 29, 35, 39	29 ± 8
	E	2.8×10^6	5	21, 24, 27, 27, 37	27 ± 6
	F	1.9×10^6	3	31, 44, 56	44 ± 13

[a] Lot 8652 of CGT-P was used in experiment A with the *Ro* strain and experiment A, B, and C with the *Ro/PM* strain; lot 9163, in experiments D and E with the *Ro/PM* strain; and lot 590647, in experiment B with the *Ro* strain and experiment F with the *Ro/PM* strain. Lot 8652 was characterized as a suspension of particles 1 to 105 μ in greatest dimensions; lot 9163 was a suspension of particles 26% of which were <23 μ, 54% were 23 to 34 μ, and 20% were >34 μ in greatest dimensions (average, 25 μ); and lot 590647 was a suspension of particles 25 to 50 μ in greatest dimensions (average 40 μ). Each preparation was suspended in benzyl benzoate and castor oil. For each preparation, the dose of CGT-P was equivalent to 50 mg of CGT per kg of body weight.

[b] In all cases, inoculations were carried out 7 days after dosage with CGT-P.

[c] Parasitemias of all three untreated monkeys inoculated with sporozoites of the *Ro* strain became patent on day 8 after challenge. Of the 10 untreated monkeys inoculated with sporozoites of the *Ro/PM* strain, parasitemias of 9 became patent on day 8, of 1 on day 9.

[d] These four monkeys were reinoculated with 2.9×10^6 sporozoites of the *Ro* strain on day 352 after dosage with CGT-P; parasitemias of three were patent on day 360, of one on day 366.

[e] These monkeys were splenectomized 426 days after dosage.

remaining 3 inoculated with the *Ro* strain and 10 inoculated with the *Ro/PM* strain served as untreated controls. Three preparations of CGT-P were used in these studies; included were the large-sized particle lot 8652 and the medium-sized particle lots 9163 and 590647.

Although the data on infections with the *Ro* strain are limited (Table 9), they suffice to show that CGT-P afforded a level of protection against infections with this strain not less, and possibly greater, than that provided against infections with the *B* strain. Of the seven subjects committed to experiments with the *Ro* strain, two exhibited patent parasitemias, one on day 116 and the other on day 578 after dosage. Protection was complete for at least 352 days for four of the remaining five monkeys and for at least 829 days for the fifth.

As compared with its efficacy against infections with the pyrimethamine-susceptible *Ro* strain, CGT-P provided relatively little protection against challenges with sporozoites of the pyrimethamine-resistant *Ro/PM* strain (Table 9). Thus, the mean intervals between dosage with CGT-P and patency ranged from 27 to 48 days in the six experiments with the *Ro/PM* strain and >352 and >508 days in the two experiments with the *Ro* strain. With respect to onset of parasitemia in untreated monkeys inoculated with the *Ro/PM* strain, that for recipients of CGT-P was delayed by 1 to 74 days.

(ii) Impacts of CGT-P on onset of patency, as measured by results of subinoculation of blood, and evolution of established infections in monkeys inoculated with the *Ro/PM* strain. The first of these issues was studied in 3 of the monkeys of experiment B and all 10 monkeys of experiment C (Table 9). There were 24 recipients of blood from these subjects, 5 from monkeys in experiment B and 19 from those in experiment C. Transfers from the experiment B monkeys were carried out late in the parasite-negative period at random times after dosage. Transfers from the experiment C monkeys were effected, whenever feasible, on days 17, 36, and 66 after dosage with CGT-P, times corresponding to days 2, 21, and 51 after onset of patency in the untreated controls.

Of the 24 transfers attempted in this subinoculation study, 10 gave negative and 14 gave positive results (Table 10). All negative results were obtained on transfers carried out 13 to 34 days before onset of patency. Of the 14 positive results, 8 were obtained on transfers carried out 1 to 10 days before onset of patency and 6 on transfers effected 14 to 54 days before onset of patency. Two of the latter six, those from Mmu 7 and A-3055, antedated negative results obtained in the same subjects by 20 and 7 days.

The intervals between injection of blood and onset of patency among the positive subinoculees (Table 10, column 5) merit special attention. In 5 of the 14 positive transferees,

TABLE 10. Results of subinoculations of blood from recipients of CGT-P carried out at various times during the thick-blood-film-negative period that separated challenge with sporozoites of the *Ro/PM* strain from onset of patency.

Donor		Recipients of blood from donor		
Monkey no.[a]	Day of patency after dosage with CGT-P[b]	Days before onset of patency in donor		Days to patency in positive subinoculees
		Negative subinoculees	Positive subinoculees	
Mmu 13	30	14	None	
Mnu 9	34	18	None	
Mmu 3	39	23	3[c]	5
Mmu 32	40	24	4[c]	7
Mmu 10	42	None	26,[c] 7[c]	11, 12
Mmu 37	42	26	6[c]	2
Mmu 41	44	None	28,[c] 8[c]	16, 10
Mmu 50	46	30	10[c]	10
A-3054	47	None	1	3
Mmu 23	49	33, 13	None	
A-3053	68	None	21, 14	10, 14
Mmu 7	70	34	54,[c] 3[c]	14, 5
A-3055	86	33	40	11

[a] Monkeys with A- prefixes were derived from experiment B; those with Mmu- prefixes, from experiment C (Table 9). All received CGT-P, lot 8652, in a dose equivalent to 50 mg of CGT per kg, 7 days before sporozoite challenge. Monkeys in experiment B were inoculated with 6.1×10^5 sporozoites; those in experiment C, with 5.1×10^5 sporozoites.

[b] Parasitemias of the two untreated recipients of 5.1×10^5 sporozoites and two recipients of 6.1×10^5 sporozoites became patent on day 8 after challenge. If related to onset of parasitemia in these controls, the time to patency for each treated subject is 15 days shorter than that indicated in this column.

[c] Beginning 5 to 7 days after onset of patency, the infections in these subinoculees were treated with CGT hydrochloride, 1.0 mg of CGT per kg, daily for 7 days, without any effect on the evolution of the parasitemia.

parasitemias were established 2 to 7 days after subinoculation; in the remaining 9 positives, intervals ranged from 10 to 16 days. Previous studies have shown that incubation periods of the latter length almost invariably result when inocula are at the threshold required for infection, i.e., 1 to 10 trophozoites (49). Assuming that such was the inoculum, the total numbers of merozoites (progeny of mature tissue schizonts) or trophozoites in the circulating blood of the donor could have been as few as 200 and not more than 2,000 at the time of subinoculation. If this extrapolation were to overestimate the population of merozoites to but a moderate degree, a negative result on blood transfer would not necessarily signify the absence of parasites in the blood, but rather that the population was so low that parasitemia could be verified only fortuitously by transfer of 10 to 15 ml of blood. It seems likely that CGT-P does significantly less in the way of delaying onset of parasitemia in subjects challenged with sporozoites of the *Ro/PM* strain than the results of examinations of thick blood films would suggest. They also imply that the steady release of small amounts of CGT, with maintenance of very low concentrations of this agent in the circulating blood, can significantly retard multiplication of the blood schizonts of the *Ro/PM* strain. The effectiveness of this steadily maintained low level in the sporozoite-induced disease is in marked contrast to the failure of a bolus dose of CGT hydrochloride to affect the course of the parasitemia in the positive subinoculees (Table 10, footnote *c*).

The impacts of CGT-P on the evolution of established infections was studied in all monkeys included in experiments A through F (Table 9). Whereas parasitological studies on these subjects were pursued for 15 to 24 months after onset of patency, only those events that took place during the first 30 days will be summarized here. Included are the time intervals between onset of patency (as measured by examinations of thick blood films) and attainment of initial parasite peaks, the heights of those peaks, and the cumulative parasite burdens incurred during the first 30 days after the onset of parasitemia. This burden was calculated as one-half the sum of the parasitemias on each of the 30 days. Although this procedure does not factor in changes in erythrocyte numbers, it provides a reasonable approximation of parasite burden in malarias of tertian periodicity, in which anemia is not severe (49).

Although there were substantial variations in data from different monkeys, the means of the characteristics of the parasitemias of treated and untreated subjects during the first 30 days after onset of patency differed in several respects (Table 11). Specifically, in the treated monkeys it took longer after onset of patency for parasitemias to peak, peaks were lower, and the 30-day cumulative parasite burdens were less than in untreated subjects. The differences between treated and untreated monkeys were especially striking in recipients of the large-sized particle lot of CGT-P in experiments A and B. These observations indicate that multiplication of blood schizonts of the *Ro/PM* strain is inhibited significantly when these forms are continuously exposed to relatively low concentrations of CGT such as would be derived from an output of 1.0 mg over a 24-h period (Fig. 1A and 3A).

(iii) Activity of CGT-P against a single challenge with trophozoites of the *Ro/PM* strain. A single experiment, very limited in dimensions, was carried out in an attempt to assess more directly the impact of the continuous exposure to the relatively low concentrations of CGT released from the muscle reservoir of CGT-P on the erythrocytic phase of infections with the *Ro/PM* strain. There were six monkeys, 3.0 to 3.4 kg in weight, in this study: five were given CGT-P in a dose equivalent to 50.0 mg of CGT per kg; one was given the oleaginous vehicle only. Fifteen days after dosage, each subject was inoculated intravenously with 5×10^5 trophozoites. This time of inoculation coincides with the time between dosage with CGT-P and onset of patency when the blood of untreated or vehicle-treated sporozoite-challenged controls is flooded with merozoites derived from the matured preerythrocytic stages of the parasite.

The results of this experiment show that administration of CGT-P significantly affected the evolution of infections induced with trophozoites of the *Ro/PM* strain (Table 12). In three of the recipients of this agent, the onset of patency was delayed for 16, 24, and 26 days. In two of these three, parasitemias peaked at low levels, significantly lower than the peak of the control, which was within the usual range for untreated infections with the *Ro/PM* strain (53). The onset of patency was delayed for at least 121 days in the remaining two recipients of CGT-P. It is possible that the original inoculum had been eradicated from these monkeys since each was completely susceptible to rechallenge with trophozoites on day 122. Although these data are limited, they indicate that a significant segment of the delay in onset of patency observed in monkeys inoculated with sporozoites of the pyrimethamine-resistant *Ro/PM* strain after dosage with CGT-P results from the susceptibility of the trophozoite phase of the resulting infection to sustained contact with CGT released from the tissue depot.

TABLE 11. Effects of dosage with CGT-P on the characteristics of the parasitemias that developed in monkeys challenged with sporozoites of the *Ro/PM* strain

Expt[a]	No. of monkeys in expt[b]	Mean ± SD (range)			
		Day of patency relative to day of inoculation	Peak parasitemia		Cumulative parasite burden days 1–30 after onset of patency (no. of parasites per 10^4 RBC)
			Day attained after onset of patency	No. of parasites per 10^4 RBC[c]	
A	5 (T)	41 ± 25 (19–82)	12.0 ± 4.0 (7–17)	219 ± 138 (93–428)	643 ± 295 (324–964)
B	9 (T)	35 ± 23 (9–79)	14.4 ± 4.9 (8–22)	222 ± 198 (33–600)	633 ± 482 (205–1,540)
C	10 (T)	37 ± 11 (23–63)	10.5 ± 3.1 (7–14)	532 ± 259 (210–1,140)	1,430 ± 631 (645–2,520)
D	5 (T)	22 ± 8 (14–32)	9.4 ± 1.1 (8–11)	667 ± 215 (412–904)	1,564 ± 849 (663–2,448)
E	5 (T)	20 ± 6 (14–30)	9.4 ± 1.3 (7–10)	356 ± 309 (114–880)	1,330 ± 1,286 (461–3,599)
F	3 (T)	37 ± 13 (24–49)	10.7 ± 1.2 (10–12)	572 ± 324 (356–944)	1,747 ± 1,743 (676–3,759)
A–F	10 (C)	8.1 ± 0.3 (8–9)	7.6 ± 1.1 (6–9)	679 ± 264 (338–1,000)	2,495 ± 873 (1,539–4,386)

[a] Reference should be made to Table 9 for details of the sporozoite inocula and dosage and preparations of CGT-P employed in the various experiments.
[b] T, Treated monkeys; C, untreated controls.
[c] RBC, Erythrocytes.

TABLE 12. Protection accorded by a 50-mg/kg dose of CGT-P, lot 8652,[a] against a single challenge with trophozoites of the *Ro/PM* strain

Monkey no.	Treatment status[b]	Days from inoculation to patency	Peak parasitemia	
			Days from patency to peak	No. of parasites per 10^4 RBC[c]
Mmu 21	CGT-P	17	10	179
Mmu 20	CGT-P	25	11	381
Mmu 2	CGT-P	27	8	1,030
Mmu 11	CGT-P	>122[d]		
Mmu 14	CGT-P	>122[d]		
Mmu 26	Vehicle	1	11	804

[a] Dose of CGT-P expressed as equivalent of CGT; lot 8652 was a suspension of particles 1 to 105 μ in greatest dimensions in benzyl benzoate and castor oil.
[b] Both vehicle and CGT-P in vehicle were injected 15 days before inoculation with trophozoites.
[c] RBC, Erythrocytes.
[d] Mmu 11 and Mmu 14 were rechallenged with 5×10^5 trophozoites of the *Ro/PM* strain 122 days after initial challenge, 137 days after dosage. Parasitemias were patent on day 1 after inoculation and peaked on day 10 at 615 and 535 parasites per 10^4 erythrocytes in the respective subjects.

(iv) Activities of CGT hydrochloride against infections with sporozoites of the *Ro* and *Ro/PM* strains. There is a potential conflict between the activities of CGT-P against infections with sporozoites and trophozoites of the pyrimethamine-resistant *Ro/PM* strain, described above, and the total lack of activity of CGT hydrochloride against similar infections with the pyrimethamine-resistant line of the *M* strain of the same plasmodium observed and recorded previously (55; L. H. Schmidt, personal observations). It seemed important to know whether this difference was related to use of different strains of parasite or different forms of CGT. To assist in resolving this issue, the activities of CGT hydrochloride were evaluated against both developing and established infections with sporozoites of the *Ro* and *Ro/PM* strains.

A group of 17 monkeys was used in this study, 6 for assessments of the prophylactic and therapeutic activities of CGT hydrochloride against infections with the *Ro* strain and 11 for companion assessments against infections with the *Ro/PM* strain. At the beginning of the study, five of the former group and nine of the latter were accorded treatment with CGT hydrochloride at a dose equivalent to 1.0 mg of CGT per kg of body weight, administered via stomach tube, once daily for 7 days. On the day after the seventh dose, all six monkeys assigned to the *Ro* arm of the study were inoculated with 2.0×10^6 sporozoites of the *Ro* strain; similarly, all 11 subjects assigned to the *Ro/PM* arm were inoculated with 6.1×10^5 sporozoites of the *Ro/PM* strain. These inoculations were performed 1 h after administration of an eighth dose equivalent to 1.0 mg of CGT per kg to all monkeys originally dosed with this agent; daily oral dosage at this same level was continued in these subjects for 7 consecutive days. Thick- and thin-blood-film examinations were initiated on all monkeys 7 days after sporozoite challenge and were repeated on a daily schedule until the experiment was terminated.

The above procedure provided assessments of the capacities of CGT hydrochloride to modify the preerythrocytic development of the *Ro* and *Ro/PM* strains, thereby delaying onset of patency. The eventual development of patent parasitemias in each of the 14 recipients of CGT hydrochloride also made it possible to compare the activities of this agent against established infections with the two strains. In this comparison, CGT hydrochloride was administered at daily oral doses equivalent to 0.3 mg of CGT per kg to subjects infected with the *Ro* strain and 5.0 mg of CGT per kg to those infected with the *Ro/PM* strain. Dosage was started during the ascending phase of the primary attack, when parasitemias of 10 to 50 parasites per 10^4 erythrocytes were attained and was repeated daily for 7 consecutive days.

The results of this study showed that daily oral administration of CGT hydrochloride in a dose equivalent to 1.0 mg of CGT per kg for 7 days before sporozoite challenge, the day of challenge, and for 7 days thereafter (a time coverage comparable to that provided recipients of CGT-P in other studies) effected no more than a 1-day delay in onset of patency in monkeys inoculated with sporozoites of the *Ro/PM* strain, contrasted with 12- to 20-day delays in monkeys challenged with the *Ro* strain (Table 13). Administration of daily doses of 5.0 mg of CGT (as CGT hydrochloride) per kg during the early developing phase of the primary attack was without effect on evolution of parasitemia in monkeys with established infections with the *Ro/PM* strain. Daily doses of

TABLE 13. Prophylactic and therapeutic activities of CGT hydrochloride in monkeys inoculated with sporozoites of the *Ro* or *Ro/PM* strain

Strain[a]	Prophylactic evaluation			Therapeutic evaluation					
						Response			
						Established parasitemia		Posttreatment	
	No. of monkeys inoculated	Daily dose (mg/kg)[b]	Days from inoculation to patency	No. of monkeys treated	Daily dose (mg/kg)[c]	No. cleared	Days from first dose to clearance (mean ± SD)	No. of relapses	Days from last dose to relapse (mean ± SD)
Ro	5	1.0	20, 22, 24, 25, 28	5	0.3	5	7.8 ± 0.8	5	9.2 ± 1.6
	1	0	8	0[d]	0				
Ro/PM	9	1.0	9 (all 9)	9	5.0	0	—[e]	NA[f]	NA
	2	0	8, 9	0[d]	0				

[a] Inocula were 2.0×10^6 sporozoites for monkeys inoculated with the *Ro* strain and 6.1×10^5 sporozoites for those inoculated with the *Ro/PM* strain.
[b] Dose of CGT hydrochloride, as the equivalent of CGT, was administered for 7 days before inoculation with sporozoites, on the day of inoculation, and for 7 consecutive days thereafter.
[c] Dose of CGT hydrochloride, as the equivalent of CGT, was administered once daily for 7 consecutive days.
[d] Control monkeys for prophylactic evaluation served as untreated controls for therapeutic evaluation.
[e] —, Treatment with CGT hydrochloride was without effect on parasitemia.
[f] NA, Not applicable.

0.3 mg/kg effected temporary clearance of parasitemia in all five monkeys with established infections with the *Ro* strain. Parasitemias were reestablished in the latter subjects 8 to 11 days after the seventh dose of CGT hydrochloride (Table 13). These results with the *Ro* and *Ro/PM* strains, paralleling those of the much earlier studies on the *M* and *M/PM* strains (55), indicate that the delays in onset of patency encountered in recipients of CGT-P challenged with the *Ro/PM* strain are not unique to this strain, but rather can be ascribed to delivery of CGT in a repository slow-release form.

Activities of DADDS alone and in combination with CGT-P. The limited protection accorded by CGT-P against infections with the *Ro/PM* strain led to studies of the activities of combinations of this agent with DADDS, a sulfone derivative prepared by Elslager and co-workers in their search for a repository antimalarial agent (19). This approach rested on the results of investigations by Thompson which showed that the activities of repository preparations of CGT-P against infections with a strain of *P. berghei* highly resistant to CGT hydrochloride and pyrimethamine could be enhanced 30- to 60-fold by coadministration with an equal amount of DADDS (61). To place the performance of the combination in perspective, our studies started with evaluations of the activity of DADDS alone and then moved on to appraisals of the activity of combination regimens.

(i) Efficacy of DADDS against single challenges with sporozoites of the *Ro* and *Ro/PM* strains. This issue was pursued in three experiments involving side-by-side evaluations of the activities of various doses of DADDS against infections with the *Ro* and *Ro/PM* strains and in three involving infections with the *Ro/PM* strain only. These six experiments were served by 83 monkeys: 17 recipients of DADDS and 5 untreated controls challenged with the *Ro* strain, and 50 recipients of DADDS and 11 untreated controls challenged with the *Ro/PM* strain. The doses of DADDS, expressed as equivalent of dapsone, ranged from 3.125 to 100.0 mg/kg and were administered 7 days before sporozoite challenge.

The results of these experiments (Table 14) show that in all essential respects the capacities of DADDS to modify infections with the *Ro* and *Ro/PM* strains were indistinguishable. They also show that none of the doses of DADDS had a significant effect on the prepatent interval. Parasitemias of 64 of the 67 recipients of DADDS became patent on either day 8 or day 9 after sporozoite challenge, as they did in the untreated controls. There were delays of 2, 2, and 12 days, respectively, in onset of parasitemia in the three remaining treated subjects, two the recipients of doses of 50.0 mg/kg and the other the recipient of 100.0 mg/kg, all inoculated with the *Ro* strain. As will be evident from observations summarized in the terminal paragraph of this section, these delays in onset of patency could have been due to the action of DADDS on the erythrocytic forms of the parasite rather than on the developing exoerythrocytic stages.

In contrast to its lack of effect on preerythrocytic development of parasites of the *Ro* and *Ro/PM* strains, DADDS, at all doses in excess of 3.125 mg/kg, significantly affected the course of the infection subsequent to onset of parasitemia. The overall effects of dosage with this agent were to delay the time at which parasitemia peaked, lower the height of the initial peak, and reduce the parasite burden during the early course of the developed infection, especially during the first 30 days (Table 14, columns 6 to 11). Although the dimensions of these effects were dose related, there were substantial animal-to-animal variations at each dose, making for data overlap at even widely separated doses. Comparison of times between onset of patency and initial peak in parasitemia of the *Ro/PM* infections among recipients of 6.25- and 100.0-mg/kg doses of DADDS serves to illustrate this variability (Table 14, columns 6 and 7). The means for this parameter of the respective groups were 48 and 171 days. Times contributing to the mean of recipients of 6.25-mg/kg doses ranged from 16 to 88 days, whereas those for recipients of 100.0-mg/kg doses ranged from 28 to 338 days.

One of the most striking impacts of dosage with DADDS on parasitic events was the reduction of parasitemia to thick-blood-film-negative status shortly after onset of patency and the maintenance of parasitemia at or close to this level for up to 5 months. This phenomenon occurred in some but not all recipients of a given dose, but was most common and of greatest dimensions at doses of 50.0 and 100.0 mg/kg. Examples are presented in Fig. 4, which charts parasitic events in pairs of recipients of 6.25, 25.0, and 100.0 mg of

TABLE 14. Protection accorded by various doses of DADDS against a single challenge with sporozoites of the *Ro* or *Ro/PM* strain

Strain[a]	Dose of DADDS (mg/kg)[b]	No. of monkeys inoculated	Days from inoculation to patency		Peak parasitemia				Cumulative no. of parasites per 10^4 RBC[c] for 30 days after onset of patency	
					Days after onset of patency		No. of parasites per 10^4 RBC			
			Mean	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Ro	3.125	3	8	8	7 ± 2	6–9	604 ± 166	412–707	1,460 ± 118	1,353–1,587
	12.5	3	8	8	17 ± 10	7–27	447 ± 216	306–696	1,184 ± 411	880–1,652
	50.0	6	9.2	8–11	43 ± 16	25–62	213 ± 128	22–337	235 ± 360	5–821
	100.0	5	10.8	8–21	103 ± 78	26–215	66 ± 54	9–141	35 ± 49	3–121
	0	5	8.2	8–9	9 ± 2	7–12	733 ± 220	413–992	2,916 ± 867	1,600–3,629
Ro/PM	3.125	3	8	8	9 ± 1	8–9	312 ± 50	258–357	1,728 ± 428	1,315–2,169
	6.25	5	8	8	48 ± 28	16–88	212 ± 87	88–309	258 ± 528	7–1,202
	12.5	8	8	8	97 ± 80	10–227	204 ± 234	29–693	78 ± 129	1–368
	25.0	5	8.4	8–9	114 ± 42	62–163	58 ± 39	22–107	5 ± 4	2–11
	50.0	14	8.2	8–9	70 ± 50	22–145	198 ± 181	12–600	122 ± 195	2–657
	100.0	15	8.3	8–9	171 ± 96	28–338	210 ± 230	16–816	17 ± 44	2–175
	0	11	8	8	8 ± 2	6-13	644 ± 249	338–1,000	1,997 ± 829	1,436–4,179

[a] The data for the *Ro* strain were developed in three separate experiments; inocula contained 2×10^5, 1.1×10^6, and 1.8×10^6 sporozoites. The data for the *Ro/PM* strain were developed in six separate experimets; inocula contained 7.2×10^4, 7.3×10^5, 1.4×10^6, 1.9×10^6, 2.4×10^6, and 4.3×10^6 sporozoites.
[b] Dose, expressed as equivalent of dapsone, was administered in all experiments 7 days before sporozoite challenge.
[c] RBC, Erythrocytes.

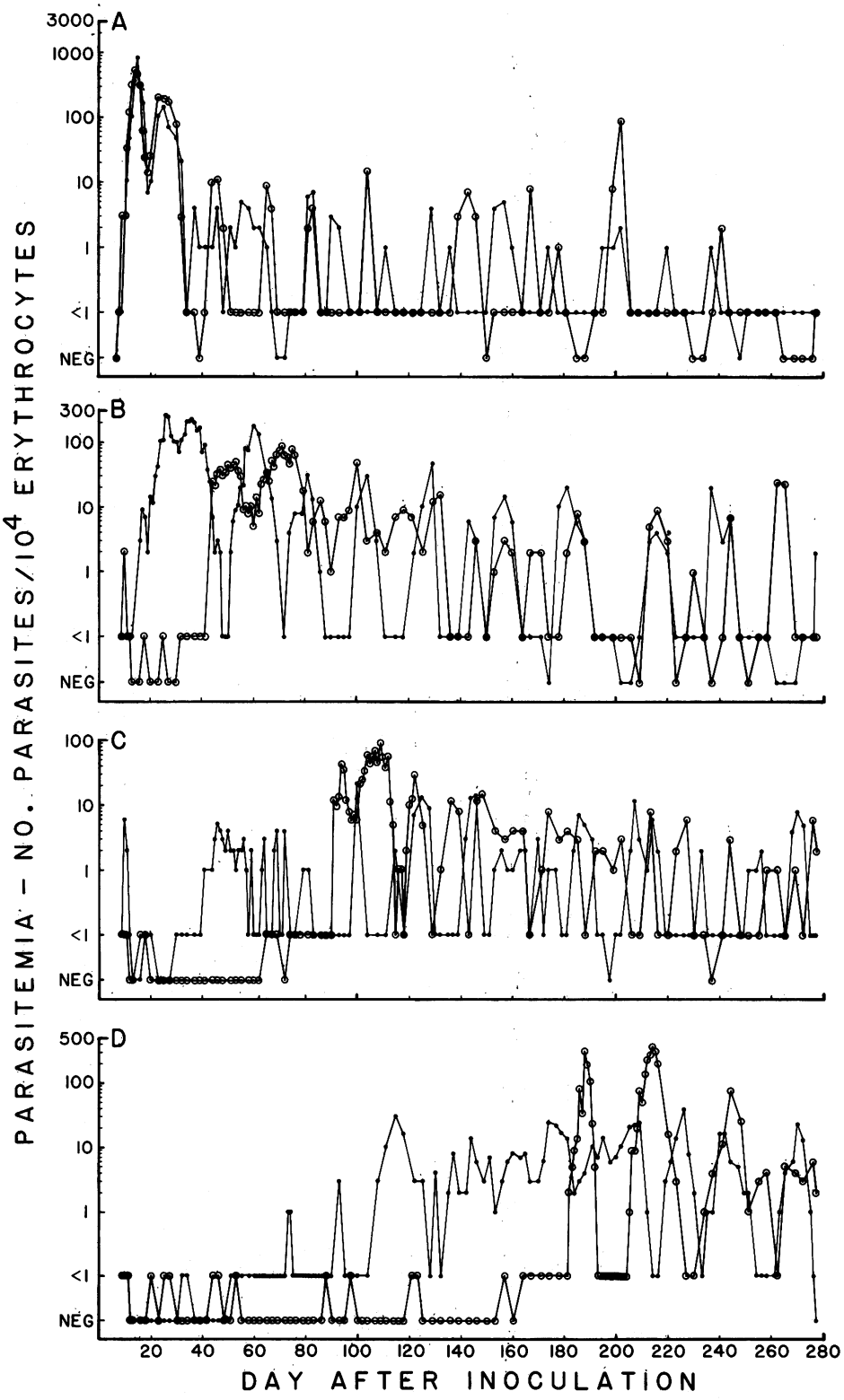

FIG. 4. Influence of dose of DADDS on evolution of parasitemia in monkeys inoculated with sporozoites of the *Ro/PM* strain. Symbols: ●, first of the monkeys in a dosage group (Table 14) to exhibit a regularly patent parasitemia; ○, last of the monkeys to exhibit regularly patent parasitemia. (A) Recipients of vehicle only. Symbols: ●, Mmu 105; ○, Mmu 130. (B) Recipients of 6.25-mg/kg doses of DADDS. Symbols: ●, Mmu 190; ○, Mmu 195. (C) Recipients of 25-mg/kg doses of DADDS. Symbols: ●, Mmu 173; ○, Mmu 161. (D) Recipients of 100-mg/kg doses of DADDS. Symbols: ●, Mmu 172; ○, Mmu 192.

DADDS per kg and two untreated controls, all infected with the *Ro/PM* strain. The pairs included those monkeys in the respective treatment groups that were first and last to develop a sustained parasitemia. Apart from showing the dimensions of this thick-blood-film-negative period and its relation to the dose of DADDS, Fig. 4 shows that once parasitemia is firmly established in recipients of this agent, it follows a course not unlike that in untreated controls.

(ii) Comparative efficacies of DADDS against challenges with trophozoites and sporozoites of the *Ro/PM* strain. The observations summarized above suggest that DADDS has little, if any, activity against the primary or secondary tissue stages of the *Ro* or *Ro/PM* strain of *P. cynomolgi*, but does have a substantial and sustained capacity to inhibit development of erythrocytic parasites. It is difficult to assess the dimensions of this latter capacity in sporozoite-induced infections because of sequential release from the primary and secondary tissue stages of progeny capable of establishing or reestablishing the erythrocytic phase of the infection. To obtain a less complicated view of the potential of DADDS as a blood schizonticide, the activity of this agent was evaluated directly against infections induced with trophozoites of the *Ro/PM* strain. To ensure interexperiment comparability, the appraisal included side-by-side assessment of the activity of DADDS against infections induced with sporozoites of this strain.

This study was served by 28 monkeys: 24 recipients of DADDS in a dose equivalent to 50.0 mg of dapsone per kg and 4 untreated controls. A feature of the experiment was the injection of DADDS at staggered intervals before challenge so as to evaluate the effects of time between dosage and challenge on activity and ensure, insofar as possible, that the intervals between dosage and onset of patency were identical in sporozoite-induced and trophozoite-induced infections. To achieve this, four groups, each of six monkeys, were injected with DADDS on day 0 or 28, 37, or 44 days thereafter. Fifty-one days after delivery of the day 0 dose, subgroups of three monkeys from each of the four treatment groups, plus two untreated monkeys, were challenged with sporozoites of the *Ro/PM* strain. Eight days later, the three remaining monkeys from each treatment group and the two remaining untreated controls were challenged with trophozoites of this strain. Blood film examinations on all 28 monkeys were initiated 2 h after trophozoite challenge and were repeated daily for at least 90 days thereafter and subsequently every other day to the end of the study.

TABLE 15. Impacts of dosage with DADDS on the evolution of infections induced by inoculation with sporozoites or trophozoites of the *Ro/PM* strain

Inoculum	Days from dosage to inoculation[a]	Monkey no.	Primary attack				Recrudescence or rechallenge		
			Days from inoculation to patency	Peak parasitemia		Cumulative no. of parasites per 10^4 RBC for 30 days after onset of patency	Day after first inoculation (day after dosage)	Peak parasitemia	
				Day after onset of patency	No. of parasites per 10^4 RBC[b]			Day after onset of patency	No. of parasites per 10^4 RBC
1.44×10^6 sporozoites[c]	0[d]	329	8	7	465	1,429			
	0[d]	331	8	7	635	1,300			
	51	202	8	12	43	156			
	51	217	8	42	408	70			
	51	226	8	45	345	11			
	23	209	8	27	509	645			
	23	214	8	26	318	631			
	23	215	8	26	354	640			
	14	293	8	37	10	44			
	14	314	8	23	56	156			
	14	319	8	59	105	257			
	7	326	9	22	117	318			
	7	327	9	22	35	83			
	7	330	8	66	22	20			
5×10^5 trophozoites	0[e]	309	3	9	570	1,442			
	0[e]	310	2	11	792	2,972			
	59	234	3	None; neg. TBF[f] days 6–44 p.i			45 (104)	15	135
	59	243	3	None; neg. TBF days 5–192 p.i.			192 (251)[g]	7	1,089
	59	244	3	None; neg. TBF days 9–31 p.i.			32 (91)	20	220
	31	219	3	None; neg. TBF days 5–192 p.i.			192 (223)[g]	11	202
	31	220	3	None; neg. TBF days 7–65 p.i.			66 (97)	12	8
	31	231	3	None; neg. TBF days 5–192 p.i.			192 (214)[g]	10	530
	22	321	2	None; neg. TBF days 4–192 p.i.			192 (214)[g]	9	1,050
	22	325	3	None; neg. TBF days 7–38 p.i.			39 (61)	21	17
	22	332	3	None; neg. TBF days 5–51 p.i.			52 (74)	11	22
	15	334	3	None; neg. TBF days 4–192 p.i.			192 (207)[g]	7	700
	15	339	3	None; neg. TBF days 4–192 p.i.			192 (207)[g]	9	515
	15	343	3	None; neg. TBF days 6–34 p.i.			35 (50)	17	13

[a] Doses of DADDS, equivalent to 50 mg of dapsone per kg, were administered intramuscularly.
[b] RBC, Erythrocytes.
[c] All monkeys in this segment of the experiment were still in the primary attack phase of the infection 192 days after sporozoite challenge, on which day they were started on a radical curative course of chloroquine plus primaquine.
[d] Untreated control monkeys.
[e] Untreated control monkeys. These animals were treated with chloroquine for cure on day 192 of the primary attack.
[f] TBF, Thick blood films; p.i., postinoculation.
[g] Rechallenged with 5×10^5 trophozoites 192 days after the initial challenge, 28 days after splenectomy.

TABLE 16. Comparison of the protection accorded by CGT-P and DADDS, administered singly and in combination, against single challenges with sporozoites of the *Ro* or *Ro/PM* strain

Strain	No. of sporozoites in inoculum	Dose (mg/kg)[a]		No. of monkeys	Days from inoculation to patency[b]
		CGT-P[c]	DADDS[d]		
Ro[e]	1.8×10^6	50	0	3	109, 571, >822
		0	50	3	9, 11, 11
		25	25	3	91, 137, 172
		50	50	3	347, >868,[f] >868[f]
		0	0	2	8, 8
Ro/PM[e]	1.9×10^6	50	0	3	24, 37, 49
		0	50	3	8, 8, 8
		25	25	3	29, 30, 31
		50	50	3	30, 56, 62
		0	0	2	8, 8

[a] Doses administered 7 days before inoculation with sporozoites.
[b] Patency was equated to detection of parasites upon up to a 20-min search of thick blood films.
[c] Dose of CGT-P expressed as equivalent of CGT; lot 590647 was used in this experiment; see Table 9, footnote *a*, for characteristics of this lot and vehicle in which preparations were suspended.
[d] Dose of DADDS expressed as equivalent of dapsone.
[e] Data on recipients of CGT-P alone and untreated controls were included in data summary in Table 9; data on DADDS alone were included in data summary in Table 14.
[f] These monkeys had been splenectomized on day 419 after inoculation.

The overall effects of dosage with DADDS on the course of sporozoite-induced infections in this experiment (Table 15) were closely comparable to the effects produced by the same dose in the preceding study (Table 14). The impacts of dosage 7 days before sporozoite challenge differed little, if at all, from those of dosage 51 days before challenge. Administration of DADDS was without effect on the onset of patency, which occurred 8 days after sporozoite inoculation in the two untreated controls, on this same day in 10 of 12 recipients of DADDS, and on day 9 after challenge in the remaining 2. As in the preceding experiment, the evolution of the established infection was modified in recipients of DADDS. Thus parasitemias were reduced to the barely thick-blood-film-positive or -negative levels shortly after onset of patency and held there for varying periods; the intervals between onset of patency and first parasite peak were prolonged; the heights of this first peak were usually much lower than those of the untreated controls; and the cumulative parasite burdens for the 30 days subsequent to onset of patency were much less than those of the controls. As in the preceding study, animal-to-animal variations in the dimensions of the effects of DADDS on established infections were considerable.

The impacts of DADDS on infections induced with trophozoites of the *Ro/PM* strain were substantially different from those just described. As with sporozoite-induced infections, the interval between dosage and challenge was not a determinant of events. Based on results of examinations of thick blood films, parasitemias of one recipient of DADDS and one untreated control became patent 2 days after challenge; those of the remaining 11 recipients of DADDS and the second untreated control became patent 3 days after challenge (Table 15). The parasitemias of both control monkeys developed in a manner normal for the *Ro* or *Ro/PM* strain (49, 53). In contrast, parasitemias of all 12 recipients of DADDS declined promptly, thick blood films becoming negative 1 to 6 days after onset of patency (4 to 9 days after inoculation). In six of these monkeys, patency was reestablished 22 to 58 days later, 50 to 104 days after dosage with DADDS. In these subjects, parasitemias peaked 11 to 21 days later than in the untreated controls and at levels approximately 1/10th those of the latter subjects.

Blood films of the remaining six recipients of DADDS were consistently negative for at least 192 days after inoculation. These subjects had been splenectomized on day 164 after challenge (179 to 223 days after dosage with DADDS) without evoking recrudescence; 28 days thereafter, they were reinoculated with trophozoites in an effort to determine whether their blood-negative status reflected the continuing output of DADDS from the muscle depot in amounts sufficient to hold parasitemia below levels detectable on thick blood films. The responses to rechallenge provided no support for this possibility, but rather testified to eradication of all parasites in the initial inoculum. Parasitemias became patent 2 to 4 days after challenge and (Table 15, columns 9 and 10) developed normally, peaking in 7 to 11 days (mean, 8.8 days) at levels ranging from 202 to 1,089 parasites per 10^4 erythrocytes (mean, 681). Except for the low peak in monkey 219, these peaks are comparable to those attained in untreated infections with the *Ro/PM* strain in subjects with intact spleens (58), although somewhat lower than those found in splenectomized subjects (L. H. Schmidt, unpublished observations).

(iii) Comparative efficacies of combinations of CGT-P and DADDS against challenges with sporozoites of the *Ro* and *Ro/PM* strains. This issue was examined in a single experiment in which the activities of CGT-P, DADDS, and combinations of these agents against infections with sporozoites of the *Ro* and *Ro/PM* strains were evaluated side by side. The experiment was served by 28 monkeys: four groups of 6 monkeys dosed with the above compounds and one group of 4 untreated controls. Each subject within a dosage group received either CGT-P at a dose equivalent to 50.0 mg of CGT per kg, DADDS at a dose equivalent to 50.0 mg of dapsone per kg, CGT-P plus DADDS in a dose equivalent to 25.0 mg of each agent per kg, or CGT-P plus DADDS in a dose equivalent to 50.0 mg of each agent per kg. A medium-sized particle preparation of CGT-P, selected to match the particle size of the DADDS preparation, was used in this evaluation. Seven days after dosage, subgroups of 12 treated and 2 untreated subjects were challenged with sporozoites of either the *Ro* or *Ro/PM* strain.

Predictably, CGT-P alone provided greater protection against infections with the *Ro* strain than against infections with the *Ro/PM* strain (Table 16). Thus, the onset of patency was delayed 101, 563, and >814 days in subjects challenged with the *Ro* strain and 16, 29, and 41 days in those challenged with the *Ro/PM* strain. Also in keeping with results of previous studies, the activities of DADDS alone against infections with the *Ro* and *Ro/PM* strains were similar (Table 16). Parasitemias of treated monkeys challenged with sporozoites of the *Ro* strain became patent 1, 3, and 3 days after onset of patency in the untreated subjects. Parasitemias of both treated and untreated monkeys challenged with the *Ro/PM* strain became patent on day 8 after inoculation. Evolution of parasitemias in recipients of CGT-P followed the diverse patterns previously described for infections with the *Ro* and *Ro/PM* strains (see above). As noted previously (see above), the evolution of parasitemia in recipients of DADDS was essentially identical in monkeys inoculated with sporozoites of the *Ro* and *Ro/PM* strains.

The protection accorded by the dosage combinations of 25 mg of CGT-P plus 25 mg of DADDS and 50 mg of CGT-P plus 50 mg of DADDS against infections with the *Ro* strain

was substantially greater than that provided against infections with the *Ro/PM* (Table 16). Surprisingly, in view of the results obtained in *P. berghei* infections in mice (61), combinations of CGT-P and DADDS did not exhibit synergistic activity. The protection accorded by the combination of 50 mg of CGT-P plus 50 mg of DADDS against infections with either strain of *P. cynomolgi* was not greater than that provided by dosage with 50 mg of CGT-P alone, whereas that accorded by the combination of 25 mg of CGT-P plus 25 mg of DADDS was clearly inferior to that provided by the above dose of CGT-P.

(iv) Expanded evaluations of efficacies of combinations of CGT-P and DADDS against challenges with sporozoites of the *Ro/PM* strain. The design of the two experiments included in this evaluation reflects an effort to improve upon the relatively poor performances of the combination CGT-P and DADDS regimens exhibited in the preceding study. In these experiments, DADDS was administered at a dose equivalent to 100.0 mg of dapsone per kg in both the mono-drug and combination regimens. The dose of CGT-P was held at 50.0 mg/kg in both regimens. In one experiment the size of the sporozoite inoculum was reduced by 1.5 logs in an attempt to lessen the burden of protection imposed on the combination regimen.

The primary purposes of these experiments were served by 39 monkeys: 10 recipients of CGT-P at a dose equivalent to 50.0 mg of CGT per kg, 10 recipients of DADDS at a dose equivalent to 100.0 mg of dapsone per kg, 15 recipients of the above doses of CGT-P and DADDS in combination, and 4 untreated controls. A preparation of CGT-P of medium-sized particles, matching the preparation of DADDS in this respect, was used in these experiments. Seven days after dosage, each of the 35 treated and 4 untreated monkeys was challenged with sporozoites of the *Ro/PM* strain. For reasons detailed later, a supplementary subinoculation component was introduced into the second experiment.

Administration of CGT-P alone effected 6- to 29-day delays in onset of parasitemia and slight reductions in the level of the initial peak in parasitemia compared with the dimensions of the same data items in untreated controls (Table 17 and Fig. 5A and B). These performances of CGT-P were not influenced by the size of the sporozoite inoculum. Overall, the accomplishments of CGT-P in this study were comparable to those attained in other experiments with preparations of medium-sized particles but, with few exceptions, were inferior to accomplishments of preparations of large-sized particles (Tables 9 and 11).

DADDS alone was without effect on the interval between sporozoite challenge and onset of parasitemia (Table 17) but, as in previous studies (Table 14), affected the subsequent evolution of parasitemia significantly. Effects included extension of the intervals between onset of patency and the initial peak in parasitemia and reductions in the parasite level during this interval and at the peak (Table 17, columns 7 to 10, and Fig. 5A and C). Although the dimensions of these effects varied considerably from monkey to monkey, they tended to be greater in subjects challenged with the smaller sporozoite inoculum. Thus, the mean intervals between onset of patency and the initial peak in parasitemia were 133 and 287 days for monkeys challenged with 4.3×10^6 and 9.1×10^4 sporozoites, respectively, whereas the mean peak parasitemias were 236 and 126 parasites per 10^4 erythrocytes for these inoculum groups.

Measured by impacts on the intervals between sporozoite challenge and onset of parasitemia and between onset and initial parasite peak and by the height of that peak, increasing the dose of DADDS from 50.0 to 100.0 mg/kg in the DADDS–CGT-P combination improved the efficacy of this regimen strikingly (cf. Tables 17 and 16). There were monkey-to-monkey variations in performance (Table 17 and Fig. 5D). None, however, resulted in overlaps with activities exhibited by mono-agent regimens. The accomplishments of the combination regimen in subjects challenged with the smaller inoculum were greater in two respects than in those challenged with the larger inoculum (Table 17). These included the delay in onset of patency, with means of 111 days in recipients of 4.3×10^6 sporozoites and 280 days in recipients of 9.1×10^4 sporozoites, and the intervals between patency and parasite peak, with means of 39 and 64 days in the recipients of the respective inocula.

As recorded in an earlier section of this report (see above and Table 10), monkeys challenged with the *Ro/PM* strain subsequent to dosage with CGT-P often carried a parasitemia so low that it could not be detected by rigorous,

TABLE 17. Protection accorded by CGT-P and DADDS, administered singly and in combination, against a single challenge with sporozoites of the *Ro/PM* strain

Expt[a]	Dose (mg/kg)[b]		No. of monkeys	Days from inoculation to patency		Peak parasitemia			
						Days after onset of patency		No. parasites per 10^4 RBC[c]	
	CGT-P[c]	DADDS[d]		Individual monkeys	Mean ± SD	Mean ± SD	Range	Mean ± SD	Range
A	50	0	5	14, 19, 21, 27, 32	23 ± 7	9 ± 2	7–11	309 ± 191	149–610
	0	100	5	8, 8, 8, 8, 8	8	133 ± 68	59–216	236 ± 232	74–638
	50	100	10	84, 92, 93, 96, 104, 105, 110, 146, 166, 196	119 ± 37	39 ± 15	19–70	145 ± 120	12–324
	0	0	2	8, 8	8	7	6, 8	736	472, 1,000
B	50	0	5	17, 17, 20, 31, 37	24 ± 9	10 ± 1	9–11	555 ± 228	292–916
	0	100	5	8, 8, 8, 8, 8	8	287 ± 51	217–340	126 ± 212	16–505
	50	100	5	>145,[f] 263, 270, 306, 312	288 ± 25	64 ± 61	20–154	316 ± 181	78–472
	0	0	2	8, 8	8	8	7, 9	944	888, 1,000

[a] Inocula were 4.3×10^6 sporozoites for experiment A and 9.1×10^4 sporozoites for experiment B.
[b] Doses were administered 7 days before inoculation with sporozoites.
[c] Dose of CGT-P expressed as equivalent of CGT; lot 9163 of CGT-P used in experiments A and B. For characteristics of this lot see Table 9, footnote *a*.
[d] Dose of DADDS expressed as equivalent of dapsone.
[e] RBC, Erythrocytes.
[f] This monkey died of dysentery on day 145 after inoculation, before exhibition of parasites on thick blood films; it was not a contributor to peak parasitemia data.

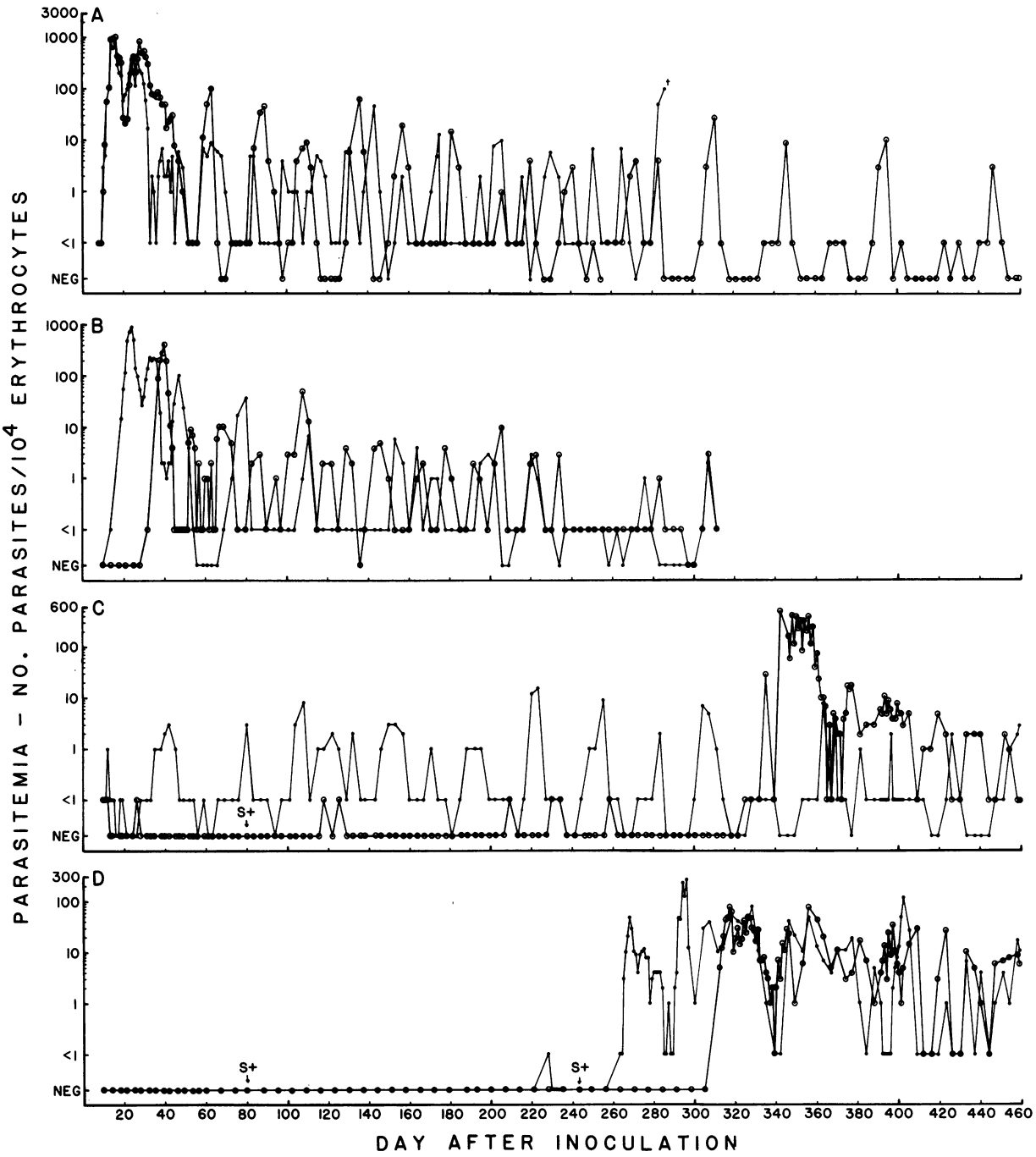

FIG. 5. Protection accorded by CGT-P and DADDS, alone and in combination, against infections with sporozoites of the *Ro/PM* strain. Symbols: ●, first of the monkeys in a dosage group (Table 17, experiment B) to exhibit regularly patent parasitemia; ○, last of the monkeys to exhibit regularly patent parasitemia; †, death from dysentery; S+, positive blood subinoculation. (A) Recipients of vehicle only. Symbols: ●, Mmu 16; ○, Mmu 45. (B) Recipients of CGT-P equivalent to 50 mg of CGT per kg. Symbols: ●, Mmu 36; ○, Mmu 8. (C) Recipients of DADDS equivalent to 100 mg of dapsone per kg. Symbols: ●, Mmu 19; ○, Mmu 18. (D) Recipients of CGT-P equivalent to 50 mg of CGT per kg plus DADDS equivalent to 100 mg of dapsone per kg. Symbols: ●, Mmu 38; ○, Mmu 39.

repetitive searches of thick blood films but could often be identified by blood transfer. This observation was at the root of the incorporation of a subinoculation component into the second experiment in this series, that employing the smaller sporozoite inoculum. This component involved transfer to 19 clean monkeys of blood drawn from four of the five recipients of DADDS (the fifth was excluded because of nearly continuously positive thick blood films) and all five recipients of CGT-P plus DADDS during thick-blood-film-negative intervals.

The results of these blood transfers show that establishment of parasitemia was delayed in the recipients of the CGT-P–DADDS combination but suggest strongly that there was a persisting low-level parasitemia during the ensuing

136- to 303-day periods of thick blood film negativity encountered in these subjects (Table 18). Thus, none of five transfers of blood on day 9 after sporozoite inoculation (the day after onset of patency in the untreated controls) was infective. In contrast, five of five transfers on day 80 after sporozoite challenge and four of four on day 243 produced infections in the respective transferees. In keeping with earlier experience, four of five transfers from recipients of DADDS alone were infective, three performed on day 80 and one on day 103 after challenge.

DISCUSSION

Background. The need for a long-acting antimalarial drug that would complement the activities of residual insecticides was recognized in 1956, shortly after inception of the Malaria Eradication Program of the World Health Organization (4, 65). In late 1957, investigators in the Research Division, Parke, Davis & Co., embarked on studies designed to meet this need (19). Their efforts covered essentially all compounds then known to have unequivocal activity against malarial infections of humans or animals (19). They prepared water-insoluble salts of such agents or derivatives that could be cleaved to the parent compound by enzymatic processes, administered these products parenterally to mice, and determined the duration of protection provided by such dosage against challenges with trophozoites of *P. berghei*. CGT-P was the most promising agent to emerge from these studies. It had been prepared in the late 1950s (E. F. Elslager and D. F. Worth, U.S. patent 3,074,947, January 1963) and evaluated for potential as a long-acting antimalarial agent by the late Paul Thompson and co-workers (60). These evaluations showed that single subcutaneous doses of 24 to 1,317 mg of CGT-P per kg, suspended in either an oleaginous or an aqueous vehicle, protected mice against a single challenge with trophozoites of *P. berghei* for 1 to 16 weeks after dosage. Supplemental appraisals showed that intramuscular doses of 38 to 400 mg of CGT-P per kg, suspended in either vehicle, protected rhesus monkeys against either single or repetitive challenges with trophozoites of the *B* strain of *P. cynomolgi* for 5 to 94 weeks after dosage.

In late 1961, Thompson turned to us for assessments of the capacity of CGT-P to protect rhesus monkeys against infections with sporozoites of *P. cynomolgi* and to G. Robert Coatney, National Institute of Allergy and Infectious Dis-

TABLE 18. Results of subinoculations of blood from recipients of DADDS or DADDS in combination with CGT-P carried out during thick-blood-film-negative intervals that preceded establishment of regularly patent parasitemias

Donors inoculated with sporozoites of the *Ro/PM* strain[a]						Subinoculees		
	Dose (mg/kg)[b]		Day of subinoculation			Day of patency after subinoculation	Peak parasitemia	
Monkey no.	CGT-P[c]	DADDS[d]	After sporozoite inoculation	After last pos. TBF[e]	Before next pos. TBF		Day attained after onset of patency	No. of parasites per 10^4 RBC[f]
18	0	100	80	54	38	8	8	590
25	0	100	80	37	67	5	9	918
29	0	100	80	17	24	4	10	436
30	0	100	80	65	15	—[g]	—	—
			103	8	142	7	8	714
27[h]	50	100	9	PN[i]	>136	—	—	—
			80	PN	>65	5	9	525
38	50	100	9	PN	254	—	—	—
			80	PN	183	4	9	791
			243	PN	20	6	10	1,080
39	50	100	9	PN	297	—	—	—
			80	PN	226	5	10	742
			243	PN	63	6	9	1,107
46	50	100	9	PN	303	—	—	—
			80	PN	232	7	8	880
			243	PN	69	6	13	920
47	50	100	9	PN	261	—	—	—
			80	PN	190	6	8	749
			243	PN	27	6	10	1,070

[a] Size of inoculum was 9.1×10^4 sporozoites.
[b] Single doses of DADDS or DADDS plus CGT-P were administered 7 days before inoculation with sporozoites.
[c] Dose of CGT-P expressed as equivalent of CGT; lot 9163 was used in this study; for characteristics of this lot and suspending vehicle, see Table 9, footnote *a*.
[d] Dose of DADDS expressed as equivalent of dapsone.
[e] pos., Positive; TBF, thick blood film.
[f] RBC, Erythrocytes.
[g] —, Infection not patent.
[h] Mmu 27 died of dysentery (*Shigella flexneri*) on day 145 after inoculation.
[i] PN, Parasitemias, as detectable on examinations of thick blood films, were negative throughout subinoculation interval.

eases, Bethesda, Md., for similar evaluations in human volunteers challenged with sporozoites of *P. vivax*. Our initial studies showed that a single intramuscular dose of CGT-P, equivalent to 50 mg of CGT per kg, provided full protection for 179 to 306 days against 6 to 11 successive challenges with sporozoites of the *B* strain carried out at approximately monthly intervals starting 7 days after dosage (56). The initial studies of Coatney and co-workers showed that CGT-P in a single intramuscular dose equivalent to 5.0 mg of CGT per kg protected human volunteers for 169 to 426 days against 1 to 10 randomly spaced challenges with sporozoites of the Chesson strain of *P. vivax* (13).

Studies on infections with *P. cynomolgi*. The above demonstrations of the capacity of CGT-P to modify the courses of naturally acquired infections with *P. cynomolgi* and *P. vivax* led to the series of experiments detailed in this report. The most important results of these investigations and their implications have been summarized below.

(i) Protection accorded by CGT-P against infections with drug-susceptible strains. The major studies in this area dealt with the capacity of a fixed dose (equivalent to 50 mg of CGT per kg) of an oleaginous suspension of a predominantly large-sized particle preparation of CGT-P to provide protection against repeated or single challenges with sporozoites of the *B* strain and to control parasitemia and modify relapse patterns of established infections with this parasite. Studies of the capacity of a medium-sized particle preparation to provide protection against repeated challenges with sporozoites of the *B* strain were also undertaken, a turn stimulated by difficulties encountered in administering the large-sized particle preparation to human volunteers (13; P. Thompson and K. Courtney, Research Division, Parke, Davis & Co., personal communication). Although limited in scope, these latter studies were of special importance, for they were the only ones executed that dealt with the impacts of dosage and composition of the suspending vehicle on the duration of protection accorded by CGT-P. In addition to the above assessments against infections with sporozoites of the *B* strain, there were single evaluations of the activities of each preparation against infections with sporozoites of the *Ro* strain.

The results of the repeated-challenge studies, all involving work with the *B* strain, showed that (i) a single dose (equivalent to 50 mg of CGT per kg) of the large-sized particle preparation provided full protection against a primary challenge with sporozoites 7 or 21 days after dosage and from 2 to 10 subsequent challenges over periods ranging from 180 to 537 days (mean, 296 days); (ii) the protection accorded by the same dose of the medium-sized particle preparation, suspended in either an aqueous or an oleaginous vehicle, was substantially less than the above (mean, 167 days); (iii) the duration of protection accorded by the medium-sized particle preparation was directly related to dose, ranging from a mean of 34 days at a dose of 3.125 mg/kg to 167 days at a dose of 50 mg/kg; and (iv) the composition of the suspending vehicle did not affect the activity of the medium-sized particle preparation either significantly or consistently.

The results of the single-challenge studies showed that (i) administration of a 50-mg/kg dose of the large-sized particle preparation 7 days before inoculation with sporozoites of the *B* strain effected a 44- to 403-day delay (mean, 232 days) in onset of parasitemia in 35 monkeys and in 18 others prevented appearance of parasitemia during postinoculation observation periods of 306 to 566 days; (ii) two of the latter 18 subjects were totally refractory to rechallenge with sporozoites on day 313 after dosage, whereas six others developed smoldering, low-level parasitemias after rechallenge on that day, responses indicative of continuing release of CGT from the CGT-P depot in amounts sufficient to completely or partially suppress development of parasitemia; (iii) 8 of the above 18 subjects were fully susceptible to rechallenge 403 to 563 days after dosage, suggesting complete eradication of the initial challenge inoculum, probably suppressive cures (14, 49), and testifying to either reduction in output of CGT from the tissue depot to levels below those required for inhibition of parasite growth or complete exhaustion of the depot; and (iv) the protection accorded by 50-mg/kg doses of either the large- or medium-sized particle preparation of CGT-P against challenge with sporozoites of the *Ro* strain was at least as great, and probably greater, than that attained in comparable evaluations against the *B* strain, thereby indicating that the achievements of this triazine could not be attributed to use of a parasite uncommonly susceptible to CGT.

The results of studies on the activity of CGT-P against established sporozoite-induced infections showed that administration of a dose of the large-sized particle preparation equivalent to 50 mg of CGT per kg effected clearance of parasitemia in 7 to 8 days and delayed relapse for 194 to 530 days. The mean time to relapse, 288 days, was remarkably similar to the mean duration of protection accorded against single and multiple sporozoite challenges, at least 300 and 296 days, respectively.

Two ancillary studies yielded noteworthy results. One involved periodic subinoculations of blood from both singly and multiply challenged monkeys, the most sensitive method available for detecting the presence of parasites in the general circulation. The results certified to the absence of parasitemia at essentially all times during the protracted thick-blood-film-negative periods up to 16 days before onset of patency and sometimes later, even when, as with the multiply challenged subjects, subinoculations were carried out on the day equivalent to the onset of patency, when flooding of the blood with progeny of the developed tissue stages would be anticipated.

The second study dealt with the responses to treatment with CGT hydrochloride of infections that developed in recipients of CGT-P or in subinoculees of such recipients. The results, showing that CGT was as effective against such infections as it was against comparable infections produced by the parent strain, provided no support for the concern that emergence of parasites resistant to CGT would be an unwanted sequel to application of CGT-P.

(ii) Dynamics of release of CGT from CGT-P depots. Results of earlier studies on the excretion of CGT in urine after dosage of monkeys with either chlorguanide hydrochloride or CGT hydrochloride (54, 57) suggested that it should be possible to determine whether the levels of protection accorded by various doses and preparations of CGT-P were related to the output of CGT in urine. The recovery of this moiety from urine was first studied in nonchallenged recipients of CGT-P in doses of the large-sized particle preparation equivalent to 50 mg of CGT per kg and in doses of the medium-sized particle lots equivalent to 3.125 to 100 mg of CGT per kg. The results showed that (i) the output of CGT in urine in the first 20 days after dosage, and especially in the first 5 days, was substantially greater in recipients of the medium-sized particle preparation of CGT-P than in the recipients of the larged-sized particle preparation; (ii) as a corollary to the above, the time over which measurable amounts of CGT were found in urine after a 50-mg/kg dose of

the large-sized particle preparation of CGT-P was more than twice that after the same dose of the medium-sized particle preparation; (iii) the duration of elimination of CGT was directly related to the dose of the medium-sized particle preparation; (iv) the fraction of the total dose of CGT-P that could be recovered from the urine as CGT was independent of both dose and particle size; and (v) there were substantial differences in the rates of elimination of CGT among recipients of the same dose and preparation of CGT-P. These observations do much to explain and reconcile the differences in duration of protection provided by the large- and medium-sized particle preparations, and by different doses of the latter preparation, as well as the variations in protection encountered in different recipients of the same dose of either of these preparations.

The relations between output of CGT and duration of protection were studied directly in subjects challenged with sporozoites 7 days after administration of the large-sized particle preparation of CGT-P in amounts equivalent to 100 mg of CGT per kg via intraabdominal implant or 50 mg of CGT per kg intramuscularly. These studies showed that (i) implanted CGT-P provided full protection for a least 65 days, even though, as measured by urinary excretion of CGT, the daily release of CGT from the implant was not more than 1/10th that from the muscle depot; (ii) implant removal 15, 36, or 65 days after sporozoite challenge was followed within 7 to 8 days by onset of a normally evolving parasitemia, indicating that continuous exposure to CGT for such time periods was without effect on the viability of the preerythrocytic and developed tissue stages; (iii) within 3 to 4 days of the above implant removal times, the amounts of CGT in urine had fallen below measurable levels, an indication that the depot was the sole source of the output; and (iv) the protection accorded by dosage with CGT-P intramuscularly persisted as long as the daily output of CGT in urine approximated 0.04 mg or more. This amount equates to release, over a 24-h period, of a quantity of CGT equivalent to a dose of 0.015 mg/kg, 1/40th of the dose required for a similar level of protection when CGT hydrochloride is administered as a bolus intravenously (55).

(iii) Impacts of pyrimethamine resistance on the activity of CGT-P. The possibility that preexisting resistance to pyrimethamine would be an obstacle to broad use of CGT-P as a repository antimalarial agent was of concern at the very beginning of our studies. Hence, as soon as it was clear that CGT-P had promising activity against infections with drug-susceptible strains, this possibility was evaluated via studies on infections with the *Ro/PM* strain. As shown previously (53), infections with trophozoites of this strain are completely resistant to treatment with maximum tolerated doses of pyrimethamine, 2.5 mg/kg daily and infections with sporozoites develop normally when such doses are administered in a prophylactic regimen. The evaluations of activity against infections with this resistant strain were controlled by assessments against infections with the fully pyrimethamine-susceptible *Ro* strain (50). Both large- and medium-sized particle preparations of CGT-P, at a dose equivalent to 50 mg of CGT per kg, were used in these appraisals.

The results of this study showed that the activity of CGT-P was seriously impaired by preexisting pyrimethamine resistance. Thus, the mean delay in onset of parasitemia (measured by presence of parasites on thick blood films) was but 25 days (range 1 to 74 days) in monkeys challenged with the pyrimethamine-resistant *Ro/PM* strain, compared with 404 or more days in subjects challenged with the drug-susceptible *Ro* strain. The delay in onset of parasitemia in recipients of the large-sized particle preparation was slightly, but not significantly, greater than in the recipients of the medium-sized particles.

Although the protection accorded by CGT-P against infections with the *Ro/PM* strain was clearly limited, this agent appeared to have a significant capacity to inhibit multiplication of trophozoites of this resistant strain. This was suggested by the delays in onset of patent parasitemia despite repetitively positive blood subinoculations 21 to 54 days before appearance of parasites on thick blood films (Table 10) and by the slow evolution of parasitemia after establishment of patency (Table 11). The capacity of CGT-P to inhibit multiplication of trophozoites of the *Ro/PM* strain was demonstrated unequivocally in a special study (Table 12), the results of which showed that administration of this agent 15 days before challenge with trophozoites provided full protection to two recipients and effected 16- to 26-day delays in onset of parasitemia in three others. This result is in striking contrast to the complete absence of activity of CGT hydrochloride in doses equivalent to 0.6 to 10.0 mg of CGT per kg against infections with either the *Ro/PM* or the *M/PM* strain (Table 13) (53, 55; L. H. Schmidt, personal observations). It implies that continuous exposure of trophozoites to the relatively minute amounts of CGT released from the muscle depot had an inhibitory effect on development of this parasite stage not obtainable by flash (limited-time) exposure to concentrations manyfold greater. It was this implication that encouraged pursuit of studies on combinations of CGT-P with DADDS in the hope that a companion drug would bolster the activity of CGT-P against the trophozoite phase of the disease to a level of usefulness against infections with chlorguanide- and pyrimethamine-resistant strains.

(iv) Reduction of the liabilities of pyrimethamine resistance by administration of DADDS in combination with CGT-P. As indicated previously, appraisals of the impacts of concomitant administration of DADDS on the activity of the CGT-P against infections with the *Ro/PM* strain rested on the results of Thompson's studies in mice infected with *P. berghei* (61). These showed that the activities of CGT-P against both drug-susceptible and CGT-pyrimethamine-resistant strains of this plasmodium were enhanced 30- to 60-fold by simultaneous dosage with equal amounts of DADDS. Thompson's interest in such a combination stemmed from the pioneering demonstrations by Greenberg and co-workers (23) and Rollo (42) on the capacities of sulfonamides and sulfones to enhance the activities of chlorguanide and pyrimethamine against infections with trophozoites of *Plasmodium gallinaceum* in chickens.

Since Thompson had shown that administration of DADDS in doses of 50 mg/kg provided protection for 63 to 268 days against challenges with trophozoites of the *B* strain of *P. cynomolgi* (61), evaluation of the activities of this agent alone against infections with the *Ro/PM* strain was an essential preliminary to assessments of the performance of CGT-P–DADDS combinations. In the major segment of this appraisal, monkeys were challenged with sporozoites of the above strain or the *Ro* strain 7 days after intramuscular administration of DADDS in doses equivalent to 3.125 to 100 mg of dapsone per kg.

The results of these evaluations showed that (i) irrespective of the dose of DADDS administered, infections with the *Ro/PM* strain became patent on the same day as infections in untreated controls; (ii) as measured by the interval between onset of patency and the initial peak in parasite numbers, the height of that peak, and the dimensions of parasite burden during the first 30 days after onset of patency, parasitemias

in recipients of DADDS evolved more slowly and were less intense than those of untreated controls; (iii) the mean impact of DADDS on evolution of parasitemia was dose related; (iv) the evolution of parasitemia among individuals within any dosage group was highly variable (e.g., at a dose of 100 mg/kg, the intervals between onset of patency and initial parasite peak ranged from 28 to 338 days, the height of that peak from 16 to 816 parasites per 10^4 erythrocytes); and (v) the impacts of DADDS on the evolution of parasitemia in monkeys challenged with sporozoites of the *Ro/PM* and *Ro* strains were essentially identical.

The results summarized above implied that DADDS had a significant but highly variable capacity to control development of the erythrocytic phase of infections resulting from sporozoite challenge. The results of an assessment of the activity of DADDS (in a dose equivalent to 50 mg of dapsone per kg) against infections with trophozoites of the *Ro/PM* strain supported this implication. This evaluation showed that parasitemia was suppressed for 32 to 66 days in 6 of 12 recipients of this dose and completely eliminated (equivalent to cure of infection) in the remaining 6. Studies on the fate of repository DADDS (22; A. J. Glazko, Research Laboratories, Parke, Davis & Co., personal communication), to be dealt with in more detail later, suggest that the subject-to-subject variations in the accomplishments of DADDS may be related to differences in release of dapsone from the muscle depot and the subsequent metabolism of this active moiety.

Studies of the activity of DADDS alone were followed by evaluations of the protection accorded by dosage with combinations of CGT-P and this sulfone against infections with sporozoites of the *Ro/PM* strain. The results of these appraisals showed that (i) combinations of CGT-P plus DADDS, in a dose equivalent to either 25 or 50 mg of each agent per kg, delayed the appearance of parasites on thick blood films well beyond the onset of parasitemia in the untreated controls and recipients of DADDS alone; (ii) the delays effected by these combination regimens were not greater, however, than those encountered in some recipients of doses of 50 mg of CGT-P alone per kg; (iii) an increase in the dose of DADDS to 100 mg/kg in the combination regimen had a substantial effect on the time to appearance of parasites on thick blood films, resulting in a mean delay of 158 days (range, 76 to 304), as compared with a mean of 41 days (range, 22 to 54) in the recipients of the combination of 50 mg of CGT-P plus 50 mg of DADDS per kg; and (iv) the dimensions of the delays effected by the combination of 50 mg of CGT-P plus 100 mg of DADDS per kg were inversely related to the size of the sporozoite inoculum.

Although these studies showed clearly that the liabilities of pyrimethamine resistance could be reduced significantly by increasing the dose of DADDS in the combination to the equivalent of 100 mg of dapsone per kg, the practical significance of this reduction may not be great for several reasons: (i) the protection accorded by the combination of 50 mg of CGT-P plus 100 mg of DADDS against infections with the *Ro/PM* strain was substantially less than that provided by 50 mg of CGT-P alone per kg against infections with sporozoites of the drug-susceptible *Ro* or *B* strain; (ii) the protection attained varied widely from individual to individual; (iii) the protection was superficial in that it was referable to numbers of parasites in the circulating blood rather than freedom of blood from parasites; and (iv) the dimensions of protection were influenced by the numbers of the sporozoites in the inoculum. Even though not significant from a practical viewpoint, the superior performance of the combination of 50 mg of CGT-P with 100 mg of DADDS per kg focuses attention on the need for in-depth evaluations of what is the most favorable ratio of CGT-P to DADDS in combinations of these agents.

Studies on infections with *P. vivax* and *P. falciparum* in human volunteers. The foregoing observations, together with those of Thompson et al. (60, 61), underpinned assessments of the activities of CGT-P alone and in combination with DADDS against infections with *P. vivax* and *P. falciparum* in human volunteers (8–10, 12, 13, 17, 31, 38, 39). The majority of these studies were carried out under the aegis of the National Institute of Allergy and Infectious Diseases (8–10, 12, 13, 17, 31), and a smaller but very important component was carried out under the aegis of the University of Chicago (38, 39). The highly chlorguanide- and pyrimethamine-susceptible Chesson strain of *P. vivax*, a moderately chlorguanide-resistant variant of this strain, the marginally chlorguanide-resistant Southern Rhodesian strain of *P. falciparum*, and six strains of the latter plasmodium (five of which were highly resistant to chlorguanide and pyrimethamine and one of which was moderately resistant to chlorguanide but susceptible to pyrimethamine) were used in these evaluations. A total of 104 volunteers participated directly in appraisals of the activities of CGT-P alone and in combination with DADDS. All 79 recipients of CGT-P alone received the same dose, equivalent to 5 mg of CGT per kg. Of this number, 53 received a large-sized particle preparation and 10 and 16 received medium- and small-sized particle preparations, respectively. Of the 25 recipients of the CGT-P–DADDS combination, 23 received doses equivalent to 2.5 mg of CGT plus 2.5 mg of dapsone per kg and 2 received 3.75 mg of each of the agents per kg. The major results of these evaluations have been set forth below.

(i) Protection accorded by CGT-P against infections with the Chesson strain of *P. vivax*. This investigation was focused primarily on the duration of protection accorded by CGT-P against single and repeated challenges with sporozoites. The results, to which 31 volunteers contributed, showed that (i) 13 subjects were fully protected against one to five sporozoite challenges carried out 5 to 314 days after dosage with CGT-P; (ii) the remaining 18 subjects, inoculated 1 to 10 times at 74 to 413 days after dosage with CGT-P, exhibited patent parasitemias 13 to 434 days after the last sporozoite challenge; (iii) data overlap notwithstanding, the interval between dosage with CGT-P and sporozoite challenge appeared to be an important determinant of the extent of protection provided by this agent (10 of 17 subjects challenged between days 5 and 132 after dosage being fully protected, as compared with 3 of 14 challenged between days 133 and 413); and (iv) the mean duration of protection accorded by dosage with the small-sized particle preparation of CGT-P was less by approximately 3 months than that provided by dosage with the large-sized particle preparation (16).

Additional studies on the Chesson strain, to which eight volunteers contributed, showed that dosage with CGT-P provided full protection against challenges with trophozoites and effected prompt clearance of parasitemia in volunteers with established sporozoite-induced infections (12, 13). These results, together with those of the duration-of-protection study summarized above, suggested that CGT-P would be useful in both prophylactic and therapeutic settings.

(ii) Dynamics of release of CGT from CGT-P depots. Some of the drug disposition factors that determined the duration of protection accorded by CGT-P against repetitive challenges with sporozoites of the Chesson strain were identified

through serial studies of the output of CGT in the urine of volunteers who participated in that evaluation; included were six recipients of a small-sized particle preparation and six recipients of a large-size particle preparation (16). The output of CGT in 24-h collections of urine from these volunteers, from the day of CGT-P dosage until the concentrations of the triazine fell below detectable levels, was measured via the analytical procedure described earlier in this report. These measurements showed that (i) 85 to 95% of the dose of either the small- or the large-sized particle preparation of CGT-P could be recovered in the urine as CGT equivalents; (ii) the rate of elimination varied inversely with the size of the CGT-P particles, 18.1% of the dose of the small-sized particle preparation being recovered during the first week and 47.7% during the first 4 weeks, as compared with recoveries of 6.8 and 21.8% of the dose of the large-sized particle preparation during these time periods; (iii) in keeping with this latter observation, the mean intervals between dosage with CGT-P and decline in CGT output to the minimum detectable level were 36 and 60 weeks for recipients of the small- and large-sized particle preparations, respectively; and (iv) with either preparation, the times to clearance varied widely from individual to individual (e.g., from as few as 16 to as many as 50 weeks among the six recipients of the small-sized particle preparation). Data acquired on the four recipients of the small-sized particle preparation inoculated repetitively with sporozoites were especially relevant to the mission of this study. These showed that protection against rechallenge was lost when the daily output of CGT fell below 0.25 mg. On the basis of earlier studies (57), such an output could be anticipated from administration of a bolus dose of 0.3 mg of CGT as the hydrochloride salt, equivalent to 0.0043 mg of CGT per kg for a 70-kg individual, or 0.17 mg of CGT per m^2.

(iii) Impacts of resistance to chlorguanide or chlorguanide-pyrimethamine on the activity of CGT-P. Although not so designed, the investigation of the impact of chlorguanide resistance on the activity of CGT-P had its beginnings in studies on the Southern Rhodesian strain of *P. falciparum*. These studies, to which 24 volunteers contributed, were directed toward determining the capacity of CGT-P to (i) prevent infections when administered at various times during the incubation period, (ii) provide long-term protection against sporozoite-induced infections, and (iii) eradicate blood schizonts (17, 31). The results showed that (i) CGT-P provided full protection against sporozoite challenges when administered on the day before inoculation or on days 1, 2, 3, 5, or 7 thereafter; (ii) with one exception, CGT-P provided full protection against one to six sporozoite challenges carried out 5 to 514 days after dosage, the exception being the volunteer challenged six times, the last time 446 days after administration of CGT-P; and (iii) CGT-P provided little or no protection against challenges with trophozoites 9 and 13 days after dosage.

The dichotomy between the effectiveness of CGT-P against sporozoite challenges and its ineffectiveness against trophozoite challenges was a matter of substantial concern. It could have reflected the well-recognized differences in susceptibility to chlorguanide of the preerythrocytic and erythrocytic stages of *P. falciparum* (20), differences that may have placed trophozoites beyond the reaches of the low levels of CGT that prevailed after administration of CGT-P, or it could have reflected resistance of the Southern Rhodesian strain to chlorguanide. The results of limited but carefully controlled studies on trophozoite-induced infections showed unequivocally that this strain did have a low level of resistance to the latter biguanide (17, 31). The unfavorable impact of even such a low level of resistance on the efficacy of CGT-P raised serious questions as to the usefulness of this agent in settings in which resistance to chlorguanide and to pyrimethamine was of substantial dimension.

The answer to the above question came promptly and clearly from a study of the activity of CGT-P in volunteers infected with the chlorguanide-resistant, pyrimethamine-susceptible Vietnam SN strain of *P. falciparum* (38). This study showed that challenges with sporozoites of this strain at 20 days after dosage with CGT-P evoked infections which, with respect to onset of patency, evolution of parasitemia, and intensity of symptoms of malaria, were identical with infections in untreated controls. This result was replicated in subsequent studies on five other strains of *P. falciparum* and the variant Chesson strain of *P. vivax*, each unequivocally resistant to chlorguanide, pyrimethamine, or both agents (10, 39). The composite results indicated that when administered alone, CGT-P had little future as a repository antimalarial agent in any area where there were strains of *P. falciparum* or *P. vivax* with significant levels of resistance to chlorguanide or pyrimethamine.

(iv) Protection accorded by CGT-P–DADDS combination against infection with strains of *P. falciparum* and *P. vivax* resistant to chlorguanide and pyrimethamine. The capacities of a 1:1 CGT-P–DADDS combination to prevent infection, provide long-term protection against sporozoite challenges, and eradicate the erythrocytic stages of the plasmodium were evaluated in 26 volunteers (8, 9). The Malayan III, Malayan IV, and Thai II strains of *P. falciparum* and a variant Chesson strain of *P. vivax*, all resistant to chlorguanide and pyrimethamine, were included in this appraisal. These studies produced a mix of promising and unpromising results. On the favorable side, they showed that (i) the CGT-P–DADDS combination administered on the day of sporozoite challenge provided substantial protection against infections with each of the aforementioned strains of *P. falciparum*, eight of nine volunteers being fully protected and the ninth exhibiting a slowly evolving parasitemia after a delay of 19 days in onset of patency; and (ii) the combination had a significant, although variable, capacity to control established infections with the various strains of *P. falciparum*, clearing parasitemia in 3 to 7 days in all 14 volunteers and curing infections in 9. On the unfavorable side, they showed that (i) the combination provided protection against rechallenge with sporozoites of the Malayan III strain of *P. falciparum* for a very limited time period, if any, clearly less than 70 days; and (ii) the combination failed to clear parasitemias in volunteers with established infections with the chlorguanide-resistant variant of the Chesson strain of *P. vivax*, although it restrained development of parasitemia.

Studies on the activity of CGT-P–DADDS combinations in human volunteers were terminated at this point. Those responsible for this action concluded that "because of the lack of uniform effectiveness against multiresistant falciparum malarias plus its short duration of action" the combination "has limited possibilities as an antimalarial agent" (9). This conclusion may have been correct, but given the limited dimensions of the evaluation of the combination, it may also have been premature. There had been no study of dose-response relationships in any segment of the assessment of the activity of CGT-P in human volunteers. There was no reason to consider that the 5-mg/kg dose used in the mono-drug regimen was optimal, much less supraoptimal. There was no evidence that this was the maximum tolerated dose of CGT-P; hence there was no basis for reducing the 5-

mg/kg dose by half in the combination regimen. Likewise, there was no valid basis for administering DADDS at a dose of 2.5 or, at most, 3.75 mg/kg, or for its delivery with CGT-P in a 1:1 combination. No attention had been given to the activities of preparations of different-sized particles of either CGT-P or DADDS, despite the clear superiority of the large-sized particle preparation of CGT-P in the studies on *P. vivax*. No attention had been given to the release of dapsone from DADDS or its metabolism after release to see whether there were important subject-to-subject differences, as there were with CGT-P. These issues, which can be studied critically and relevantly only in human volunteers, would have seemed worthy of investigation before bringing studies on the CGT-P–DADDS combination to a close.

(v) Comparability of therapeutic accomplishments and disposition of CGT-P alone and in combination with DADDS in human volunteers and rhesus monkeys. From a qualitative viewpoint, the performances of CGT-P alone against infections with *P. vivax* and *P. falciparum* in human volunteers and infections with *P. cynomolgi* in rhesus monkeys were remarkably similar. This similarity was commmon to infections with either drug-susceptible or chlorguanide-pyrimethamine-resistant strains, except the Southern Rhodesian strain of *P. falciparum*, for which the highly pyrimethamine-resistant *Ro/PM* strain was a poor counterpart.

From a quantitative viewpoint, the performance of CGT-P against infections with the *B* and *Ro* strains of *P. cynomolgi* underpredicted the activity of this agent against infections with the parent Chesson strain of *P. vivax*. A dose of CGT-P equivalent to 5 mg of CGT per kg (195 mg/m^2) provided protection against infections with the latter strain at least equal to, and probably greater than, that provided by a dose equivalent to 50 mg of CGT per kg (615 mg/m^2) against infections with the *B* or *Ro* strain. The results of urinary excretion studies indicated that the longer protection in human volunteers was related to slower release of CGT from the CGT-P depot (16). It is of interest, however, that protection against infections with either the Chesson strain of *P. vivax* or the *B* strain of *P. cynomolgi* was lost when, as judged by output of CGT in urine during a 24-h interval, release of this active moiety from the tissue depot fell below 0.17 to 0.18 mg of CGT per m^2. The limited dimensions of the studies on the chlorguanide-resistant variant of the Chesson strain and the chlorguanide-pyrimethamine-resistant strains of *P. falciparum* precluded quantitative comparisons of the performance of CGT-P against infections with these strains and those with the *Ro/PM* strain.

There were shortcomings in the studies on the activities of combinations of CGT-P with DADDS in human volunteers which compromise any comparison of the responses of infections with *P. vivax* and *P. falciparum* in these subjects with those of infections with *P. cynomolgi* in rhesus monkeys. The volunteer studies were very limited in dimensions, dealt only with infections with resistant strains, utilized (without a control assessment) a smaller dose of CGT-P than was employed in the single-agent regimen, and provided no information on the accomplishments of DADDS alone. These limitations notwithstanding, the results suggested that, at the doses used, the CGT-P–DADDS combination was less active against infections with the chlorguanide-resistant variant of the Chesson strain of *P. vivax* than against the *Ro/PM* strain of *P. cynomolgi*, while being more active against infections with the resistant strains of *P. falciparum*. The first of these suggestions may have been due to differences in the level of pyrimethamine resistance of these strains (53). The latter suggestion may reflect the absence of persisting tissue schizonts in infections with *P. falciparum*, plus the fact that dapsone, the cleavage product of DADDS, is more active against the blood schizonts of this plasmodium than against the blood forms of *P. cynomolgi*. To this superior activity must be added the impacts of differences in metabolism of dapsone in humans and rhesus monkeys (A. J. Glazko, Research Laboratories, Parke, Davis & Co., personal communication). Both convert this sulfone to its less active monoacetyl derivative, the monkey to a much greater extent than humans. As a result of this difference in metabolism, the ratio of dapsone to its monoacetyl derivative in plasma of humans dosed with DADDS is 1.4 compared with 0.05 in the plasma of monkeys treated similarly.

The overall performances of CGT-P alone and in combination with DADDS against infections with *P. vivax* and *P. falciparum* in human volunteers and *P. cynomolgi* in rhesus monkeys were remarkably similar. This is of importance, first because the studies in human volunteers were guided by the results of the studies on *P. cynomolgi* and second because current strictures on investigations employing human volunteers will make future searches for repository antimalarial agents and their evaluation in naturally infected humans more dependent than ever before on results obtained in the *P. cynomolgi*-rhesus monkey model or other experimental animal models with equal predictive capabilities.

Field trials on CGT-P alone and in combination with DADDS. The initial reports on the results of the studies in human volunteers which showed that CGT-P could provide long-term protection against challenges with sporozoites of *P. vivax* and *P. falciparum*, even in the face of a low level of resistance to chlorguanide, generated intense interest among those directly involved with malaria control problems and made them eager to determine whether this agent could provide the same level of protection in the field. Their interests in moving ahead at once with this determination could not be turned aside, even though, in retrospect, it might have been advisable to have deferred such trials until relations between subject activity, particle size, and dose of CGT-P and effect were better established and factored into their design. Between early 1964 and mid-1965, field trials were mounted that involved more than 6,000 participants, of whom at least 4,000 were recipients of CGT-P (Ken Courtney, Department of Clinical Investigation, Research Laboratories, Parke, Davis & Co., personal communication, 27 June 1966). By 1967 there were published reports on the results of trials carried out in Pakistan (15), New Guinea (40), Australia (1), Gambia (32), Senegal (33–37), and Tanzania (29). Results of studies pursued in Nigeria and Rhodesia were reported somewhat later (21, 25). Except for the trials in Senegal and Tanzania, all were initiated in areas where the plasmodia were known (or assumed) to be susceptible to chlorguanide and pyrimethamine. In all of the trials, CGT-P, suspended in an oleaginous vehicle, was injected intramuscularly into a buttock at a maximum dose of 350 mg, adjusted downward according to the size of the participant. All age groups were included in the trials.

The great majority of the trials were carried out in areas in which infections with *P. falciparum* predominated. In all areas, administration of CGT-P effected rapid clearance of parasitemia. In other respects, the accomplishments of this agent fell substantially below expectations. Some participants exhibited patent parasitemias as soon as 1 month after dosage, the majority within 4 months; very few were protected for as long as 7 months. In all trials, protection was of

shortest duration in children, and in all age groups, it was of shorter duration in areas where chlorguanide-pyrimethamine resistance had been identified before the trial than in areas in which there was no evidence of such resistance. In one small-scale trial (1) in which the focus of interest was on established *P. vivax* infections, the impact of dosage with CGT-P on time to relapse was significant but much less than was anticipated from the results in human volunteers. Participants in this study, in a setting where the possibilities of reinfection were excluded, exhibited patent parasitemias within 2 to 5 months of dosage.

Whereas the protection that CGT-P had provided in the trials against infections with *P. falciparum* was greater than that of any known antimalarial agent, its duration was so far short of expectations that enthusiasm for further work on this repository agent was dampened. Instead of exploring the possibilities of obtaining more from CGT-P than had been attained in the initial field studies, attention was directed to trials of combinations of CGT-P with DADDS. This turn rested on the results of the early experimental studies primarily in mice infected with *P. berghei* (61), without input from the results of studies on *P. cynomolgi* infections in rhesus monkeys and *P. falciparum* infections in human volunteers which were still in progress. Trials involving at least 2,800 participants were carried out in New Guinea (41), Australia (2), Tanzania (11, 29), Nigeria (28), and Brazil (24). In two of these evaluations, the performance of CGT-P plus DADDS was compared with that of CGT-P alone (29, 41). The maximum dose of the combination utilized in the majority of the trials was 450 mg (225 mg of each component), adjusted downward for children and infants. In a few trials, the maximum dose was 300 mg. It is worth noting that the amount of CGT-P in either of these dose combinations was less than that in the trials of this agent alone.

The results of these trials showed that the duration of protection provided by the CGT-P–DADDS combination against infections with *P. falciparum* was at best only slightly longer than that attained with CGT-P alone (29, 41). Likewise, the results of a small-scale study on established infections with *P. vivax* showed that the effects of this combination regimen on interval to relapse were no different than those of CGT-P alone (1, 2). Those responsible for these field trials saw little in the results that would justify continued investigation of either CGT-P or its combination with DADDS. Field studies on these agents came to an end in 1968.

The reaction to the outcome of the various field trials was understandable. Nonetheless, it was and is surprising that no serious efforts were made to determine why CGT-P alone or in combination with DADDS performed less well in the field than in human volunteers. One might possibly attribute these differences in performance to use of hypersusceptible test strains in the volunteer studies, exposure to different-sized sporozoite inocula in the two settings, unrecognized resistance to chlorguanide and pyrimethamine in the field, or dominance of *P. falciparum* infections in that area. There are good reasons, however, for discounting the importance of each of these factors. First, extended experience indicates that infections with the Chesson strain of *P. vivax* provide a severe test of the activity of agents in the antifol class, as do infections with the Southern Rhodesian strain of *P. falciparum*, with its low level of resistance to chlorguanide. Second, volunteers were inoculated repetitively with large numbers of sporozoites, probably heavier challenges than are attained in the field, although doubtless not as frequent. Third, the prompt clearance of parasitemia after delivery of CGT-P either alone or in combination with DADDS to actively infected participants in the field trials argues for full susceptibility to chlorguanide or pyrimethamine. And last, although the duration of protection was different, CGT-P alone or with DADDS provided essentially the same levels of protection against infections with *P. vivax* and *P. falciparum* in both field and volunteer settings.

It seems more likely that the diverse performances of CGT-P alone and in combination with DADDS in the two settings were related to nothing more than the differences in the times over which effective amounts of CGT were released from the depot of CGT-P in muscle. Studies in human volunteers (16) and rhesus monkeys (this report) have shown that duration of release is determined by particle size and dose of CGT-P, to which might be added physical activity of the recipient. The preparations of CGT-P utilized in the field trials contained small- and medium-sized particles, whereas the preponderance of those used in the volunteer studies contained large-sized particles. As judged by the results in human volunteers (16), this use of the small- and medium-sized particles, a choice based primarily on ease of administration of such preparations, could have halved the duration of protection attained at a given dose of CGT-P. Although on a milligrams-per-adult recipient basis the same dose of CGT-P was used in the volunteer and field trials, the shift from large- to medium- and small-sized particles may have resulted in a substantial decrease in "functional dose" in the latter trials. Studies in rhesus monkeys (this report) have shown that the slope of the dose-response curve for CGT-P is steep. As a result, a relatively small decrease in dose effected a striking reduction in the duration of protection. Unfortunately, dose-response relations were not studied in any human trial. If they follow the rhesus monkey pattern, as might be expected, a relatively small decrease in functional dose could decrease the duration of protection substantially. Lastly, the physical activity of the participants in the field trials was undoubtedly greater than that of the volunteers, whose activities were severely restricted. This difference might have favored both mobilization of CGT from the muscle mass and renal clearance in the field trial participants, thereby shortening via still another mechanism the time over which effective levels of CGT were maintained in the circulating blood.

Local reactions at the injection site may also have contributed to more rapid clearance of CGT in at least some participants in the field trials. Such reactions were rare in the volunteer studies, occurring in but two of the more than 100 subjects (16). In these two, however, the protection accorded against challenge with sporozoites was strikingly shorter than in other volunteers in the same study and the output of CGT in urine was accelerated greatly. For reasons that cannot be identified with certainty, the incidence of local reactions among the participants in field trials was closer to 20% than 2%. If clearance of CGT in subjects so affected paralleled that in the volunteers, a marked shortening of the duration of protection would have resulted.

It is unfortunate that there was no evaluation of the impacts of the above factors on the outcome of the clinical trials, especially since this could have been done with comparative ease. Were the factors found to be operative, appropriate modification of either the dose or dosage form (or both) of CGT-P might have extended the duration of protection significantly. In the absence of serious efforts to determine the reasons for the differences in duration of protection accorded by CGT-P in human volunteer and field settings, the essentially total loss of interest in continued

trials of this agent in areas where infections were responsive to chlorguanide and pyrimethamine may have been premature.

Directions of current endeavors. Since the renewal of interests in repository antimalarial drugs (WHO-TDR/CHEMAL/SC(33)/77.3, item 1.2, p. 1) was the catalyst for bringing together the results of our unpublished studies on CGT-P and synthesizing them with the results of studies in human volunteers and field settings, it would be inappropriate to close this report without comment on this endeavor. Although approaches to the goal have been varied, the major focus seems to have been on incorporation of an antimalarial agent into a biodegradable polymer which may be either implanted subcutaneously or injected intramuscularly as microspheres. Blood schizonticides have received the most attention, the majority antifols. Included were pyrimethamine, cycloguanil, WR-158,122 [2,4-diamino-6-(2-naphthyl)-sulfonyl-quinazoline], and WR-99, 210 (4,6-diamino-1-[(2,4,5-trichlorophenoxy)-propoxy]-1,2-dihydro-2,2-dimethyl-*s*-triazine), as single agents or in combination with a sulfonamide or sulfone. The duration of protection provided by preparations of these agents in polymers has been evaluated against infections with *P. berghei* in mice.

Although basically the concept of slow delivery of antimalarial agents via incorporation in a biodegradable polymer has much to offer, the choices of test agents and test infections and the failure to quantify rate of drug release in vivo are causes for concern. First, resistance to pyrimethamine and chlorguanide is now well established in almost all areas of malaria prevalence and is of such dimensions that it impairs the utility of these agents even when administered in combination with sulfonamides or sulfones (5). Likewise, although to a lesser degree, resistance to pyrimethamine impairs the activity of the quinazoline WR-158,122, whether delivered alone or in combination with sulfadiazine (45, 46). In view of this resistance problem, which is substantially more widespread than when CGT-P was evaluated in the field, the outlook for effective application of pyrimethamine, CGT, or WR-158,122 in biodegradable polymers (or any other carrier), with or without sulfonamides or sulfones, seems bleak.

As for WR-99,210, the results of small-scale studies in owl monkeys have shown that this triazine is equally active against infections with pyrimethamine-susceptible and -resistant strains of *P. falciparum* (44). The daily dose of WR-99,210 required for a curative result is relatively large, 10 mg/kg; furthermore, a daily dose of 1.5 mg/kg was without effect on developing parasitemia. The size of the curative dose of WR-99,210, together with its steep dose-response curve, argues against the effective use of this agent in a biodegradable polymer.

The dose limitations of WR-99,210 highlight an essential requirement for any slow-release agent, specifically that it must exhibit its activity when delivered slowly in an exceedingly small daily dose. Pyrimethamine, CGT, and WR-158,122 satisfy this requirement and would be promising candidates for incorporation into polymers but for the obstacle of pyrimethamine resistance. There is no other known antimalarial agent that satisfies this essential requirement.

The use of infections with *P. berghei* in mice as the primary test object also has some serious limitations. First, the model has not been useful for evaluating the protection accorded by any agent against repetitive challenges with sporozoites (47, 48). Second, effects of drugs against challenges with trophozoites of this plasmodium are best measured by reductions in parasitemia rather than freedom from parasitemia, which is the target of concern in the control of malarial infections in humans (60, 61). Third, although infections with *P. berghei* in mice have been of tremendous value in identifying agents with blood schizonticidal activity, they have a poor record of quantifying that activity with respect to agent performance against infections with *P. falciparum* or *P. vivax* in humans. Lastly, the size of the muscle mass in the mouse makes for difficulties in quantitative delivery of any agent intramuscularly. *P. falciparum* and *P. vivax* in owl monkeys would be ideal tools but for the difficulties in obtaining large numbers of sporozoites of *P. falciparum* on a regular schedule and the uncertainties of the course of infections induced with sporozoites of *P. vivax* (43). The best all-around tool would doubtless be *P. cynomolgi* infections in rhesus monkeys.

Lastly, there have been concerns with the failure to include measurements of the release of the test compound from the polymer in vivo as an essential element of the search for long-acting preparations. Once the daily output of agent required for protection against infection in an animal model has been established (and this could be done via either continuous intravenous infusion or use of a mini-pump), the duration of protection to be expected from application of diverse preparations should be ascertained by measuring the output of the agent in urine of noninfected humans. Demonstration of the value of this approach was one of the important contributions of the experimental animal and human volunteer components of the studies on CGT-P. Had the approach been utilized in the evaluation of CGT-P in the field, these trials might well have been more productive.

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